A TEXT-BOOK OF BIOLOGIC ASSAYS

PITTENGER

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BY

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WITH ONE HUNDRED FIFTY-THREE ILLUSTRATIONS

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PREFACE TO THE SECOND EDITION

At the time of the publication of the first edition of this text book in 1914 the subject of Biologic Assays was taught in very few Colleges of Pharmacy or Medicine and was not recognized by the United States Pharmacopoeia or the National Formulary.

Biologic Assays at that time were being conducted by only a few experts engaged in the larger laboratories devoted to drug standardization work.

The only official recognition previous to 1914 was taken by the United States Pharmacopoeial Convention of 1910 in recommending to the Revision Committee "that biological tests or assays, when accurate and reliable, may be admitted" to the U.S.P. IX. This recommendation resulted in the inclusion of several optional and two compulsory Biologic Assays in the U.S.P. IX (Official from Sept. 1, 1916 to Jan. 1, 1926).

The U.S.P. IX included the following statement in reference to Biologic Assays:

"Inasmuch as there are several drugs more or less extensively used in the treatment of disease, for which no satisfactory chemical method of assay has been developed, other assays have been introduced in which these drugs are administered to animals and the effects noted. From the results obtained from these assays the strength of the preparation is calculated, and if necessary proper corrections are made so that the preparation shall conform to the standard which has been adopted. The official drugs to which these methods of assay have been applied in the following pages, are the members of the digitalis group, cannabis and aconite and the suprarenal gland."

"Brief descriptions of the more commonly accepted methods are given here in order, first, to direct the attention of manufacturers to them; second, to ascertain the points of weakness which may exist in them; and finally, to outline methods and establish standards

which those interested may adopt should they desire to assay their products and have them conform to the standards proposed. All preparations thus assayed and made to conform to these standards may be labeled "Physiologically assayed and standardized according to the U.S.P. IX."

"In the case of Cannabis and its preparations, the biological assay standards are compulsory."

During the period in which the U.S.P. IX was official, the various biologic assay methods were improved to a point where it was considered advisable, in the interest of uniformity, to make a greater number of methods compulsory.

The U.S.P. X (Official from Jan. 1, 1926) therefore, includes compulsory biologic assays for the following drugs and preparations: Aconitine, Aconite and its tincture, Cannabis and its fluidextract, Digitalis and its tincture, Ergot and its fluidextract, Squill and its fluidextract and tincture, Strophanthus and its tincture, Solution of Epinephrine Hydrochloride, and Solution of Pituitary.

The National Formulary (Fifth Revision) includes biologic assays for Fluidextract Aconite and Fluidextract Digitalis.

Since, under the provisions of the Pure Food and Drug Act, the standards of the Pharmacopoeia and National Formulary are made Law for Interstate Commerce in drugs and medicines, the necessity for teaching the subject of Biologic Assays in all Schools of Pharmacy and Medicine is apparent.

In revising this Manual, therefore, the wants of the pharmaceutical and medical colleges and their students have been the primary consideration. As in the first edition the methods familiar to experts, but not referred to in the Pharmacopoeia or the literature with sufficient detail for students and beginners are fully described.

In order to include the many improvements in the various methods, especially as to the technical details which lead to greater accuracy, which have been developed since the publication of the first edition, it has been necessary to practically rewrite the entire book.

The chapters which were included in the first edition have been revised and enlarged, sixty four new illustrations have been included and the following new chapters added: Piscidia Erythrina, Ephe-

drine, Mydriatics, Local Anaesthetics, Vitamins, Temperature Regulation, Interpretation of Biologic Assays, Standards, Care of Animals, Arrangement of Laboratory and Records and Reports.

This edition should, therefore, adequately meet the present day requirements for a Text-Book of Biologic Assays for students of Pharmacy and Medicine and for those engaged in laboratories devoted to the biologic standardization of drugs.

At the time of the publication of the first edition the art of testing the activity of drugs upon living animals was given various titles. For example, the American Pharmaceutical Association had a Committee on "Physiologic Assays;" the Pharmacopoeial Revision Committee used the term "Biologic Assays;" the Pharmaceutical Syllabus classified this type of work under "Pharmacodynamics" and many othes used the term "Biochemic Assays."

For various reasons the author selected from the above the title "Biochemic Drug Assay Methods" for the first edition. Since, however, the Pharmacopoeial Revision Committee has officially adopted the title "Biologic Assays" and this term has come into general use for classifying this type of work. The title of this second edition has, therefore, been changed to "Biologic Assays."

In conclusion the author wishes to acknowledge his indebtedness to the Arthur H. Thomas Co. of Philadelphia, Joseph Becker of the College of Physicians and Surgeons, New York City and Geo. H. Wahmann Manufacturing Co. Baltimore, Md. for cuts of special apparatus of their manufacture; to Sharp & Dohme of Baltimore, Md., for laboratory facilities, animals, etc. and to Leola B. Pittenger and Helen L. Bunn who have assisted editorially through the book in many capacities such as typing of manuscript, reading of proof and preparing the index.

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PREFACE TO THE FIRST EDITION

This Manual of Biochemic Drug Assay Methods is intended for students of pharmacy, pharmaceutic chemistry and medicine, also for the use of experts engaged in laboratories devoted to drug standardization work.

The data has been collected from monographs, Government bulletins, papers read before medical and pharmaceutical societies, and also from laboratory notes containing the results of the author's original research and observations.

Much of the original data was previously contributed in the form of papers to several national and state medical and pharmaceutical societies, including the American Therapeutic Society, The American Medical Association, The American Pharmaceutical Association and The Pennsylvania State Pharmaceutical Association.

The authors of much of the information on the biochemic assay of drugs appearing in the literature assume that the readers are familiar with the apparatus and technique of the subject. In other words, the literature is written for experts rather than to teach beginners. There is, therefore, a field for a work explaining in detail the methods and apparatus employed for pharmacodynamic standardization. Such a volume is demanded by the rapid advance in the scientific knowledge of drugs as therapeutic agents. It is commencing to be realized by physicians that drugs should be instruments of precision. Chemical assay and standardization is sufficient to render them so when they contain active principles of such character as to permit their identification and isolation in the pure form by chemical methods. But there is another class of drugs not amenable to chemic standardization. Such drugs as digitalis, ergot, cannabis indica, etc., do not lend themselves to standardization by chemical methods. It is with this class of drugs that this volume exclusively deals.

The wants of the pharmaceutical colleges and their students have been considered. Methods familiar to experts, but not referred to

in the literature with sufficient detail for students and beginners, are fully described. Apparatus used in the pharmacodynamic laboratories of the universities both in Europe and America, is placed before the reader in the form of picture illustrations with explanations as to the use of the same. The illustrations and detailed explanation will enable the student intelligently to follow lectures and demonstrations, and will also prove useful to persons unfamiliar with the subject and unprovided with extensive reference libraries.

Judging from the want of appreciation by the medical profession of the wide variation in the therapeutic activity of drugs (see Table 1, page 6), this Manual, although intended primarily for the use of the pharmacist and pharmaceutic chemist, could be advantageously employed in teaching medical students.

In conclusion, the author takes occasion to acknowledge his indebtedness to Professor F. E. Stewart for his aid in editing the manuscript, to Professor Charles E. Vanderkleed for collaboration in original research, to Dr. Thomas Stotesbury Githens of the Rockefeller Institute for many details in technique, to the Harvard Apparatus Co. of Boston, and C. F. Palmer Co. of London, England, for cuts of special apparatus of their manufacture, and to the H. K. Mulford Company, for laboratory facilities, animals, etc.

P. S. P.

CONTENTS

CHAPTER XIII

CHAPTER XIV

CHAPTER XV

CHAPTER XVI

CHAPTER XX

CHAPTER XXI

LIST OF TABLES

ILLUSTRATIONS AND DIAGRAMS

A TEXT-BOOK OF BIOLOGIC ASSAYS

CHAPTER I

PRELIMINARY CONSIDERATIONS

(1) Introductory

The importance of assay methods as a means of securing uniformity in the action of drug preparations has never been fully appreciated. No one has questioned the necessity for preventing gross adulteration and sophistication, but the full meaning of standardization by which drugs are rendered instruments of precision is only beginning to be comprehended.

Standardization by the use of biologic methods was officially recognized for the first time in the U.S.P. IX and the N.F. IV.

It must be apparent to any thinking person that adulteration of drugs or their sophistication must of necessity materially influence their physiologic action and therapeutic value. Even the impurities resulting from the processes of preparing medicinal chemicals may seriously modify their physiologic effects. Variation in active principles in medicinal plants is another factor of importance in considering the question of standardization.

Standardization of drugs may be accomplished either by the use of chemic or pharmacodynamic methods included under the general term pharmaceutic assaying. That the *purpose* of the assay is to secure *uniformity* is admirably set forth by Vanderkleed[1] in the following words:

"*Pharmaceutic assaying* may be defined as the art of determining the amounts of medicinally active constituents of drugs and their preparations. As such it is an exceedingly important link in the chain of progress which is so rapidly bind-

[1] From an address delivered by Prof. Chas. E. Vanderkleed before the Alumni of the New York College of Pharmacy, Jan. 11, 1911.

ing together medicine and pharmacy under the inclusive science of pharmacology Progress in therapy is largely dependent upon the *uniformity* of the medicina agents which physicians employ. The absolute necessity for the standardizatio of pharmaceutic products is therefore apparent. The argument that therapeu tists must experimentally determine the proper dosage of an agent to fit the need of each particular case is no excuse for the tolerance of variation in the strength *and potency of the remedy itself; every possible variable should be eliminated in an effort to reduce therapeutics as nearly to an exact science as is possible.*

The first requisite for the chemical standardization of complex vegetable drugs and their preparations, therefore, is to know exactly what the "medicinally active constituent" or "constituents" are. For this reason it is highly important that pharmaceutic assaying be developed and improved by *the co-operation of the chemist and the pharmacologist.*

It must be remembered that the principal end to be accomplished by the assay of a preparation is to secure a *means of measuring its therapeutic efficiency.* Hence an assay fails of its purpose unless some direct and constant ratio exists between the figures obtained by the assay process and the therapeutic activity.

Having secured such a ratio, the assay process then, and only then, becomes of value. However, when a fairly constant ratio has been established between the results of an assay process and pharmacodynamic activity, such process becomes of unquestioned value—whether or not the constituent actually determined represents the entire activity of the drug, or even whether or not it be the principal medicinal constituent. The physician is not concerned directly with the actual percentage of alkaloid, or glucosid, or resin which a drug or preparation may contain: he is concerned only that the adjustment of a preparation to such a percentage of alkaloid, or glucosid, or resin guarantees to him that this preparation will, under similar conditions, produce the same therapeutic effect that any other sample similarly adjusted will produce. The fact should be emphasized, therefore, that *any assay process* or *any means of standardization* that *tends to establish a uniform ratio between results obtained and therapeutic activity* becomes at once of unquestionable value, and continues to be of value until supplanted by a new or improved process which more closely maintains this ratio."

The purpose of the pharmacodynamic assay, just as of the chemical assay, is to secure a means of measuring therapeutic activity and to make it possible to furnish *uniform* preparations. A satisfactory method which meets these requirements may or may not involve the production of physiologic reactions similar to those for which the drug is intended to be the means of producing when used therapeutically. That the effect chosen as a means of standardization does not parallel the clinical effect sought is not sufficient to condemn the method. It is only necessary that the effect chosen as an earmark is always indicative of a good quality of the drug or preparation, and

criticisms of methods on the ground that they are *toxic* methods or that the animal chosen is biologically much different from man are made only through a lack of conception of the real purpose of the physiologic test, namely, to secure *uniformity.* The determination of the real value of a drug in the treatment of disease in man is another matter entirely.

(2) Definitions

Pharmacology.—The science that treats of drugs and medicines; their nature, preparation, administration and effect; including Pharmacognosy, Pharmacy, Pharmacodynamics and Therapy-dynamics.

Pharmacognosy treats of the identification and selection of vegetable and animal drugs.

Pharmacy is the science and art of preparing, preserving, compounding and dispensing medicines.

Pharmacodynamics treats of the actions of medicines on healthy organs.

Therapy-dynamics treats of the actions of medicine on diseased organs.

Assaying consists of the quantitative determination of one or more constituents of a product.

Standardization in a general sense means any and all methods for determining and thereby improving the character, quality and strength of materia medica products.

In the specific sense it means the adoption of definite methods and standards and adjusting materia medica products thereto.

While standardization necessarily involves assaying, it also *includes final adjustment to definite strength.*

Pharmacodynamic or physiologic standardization consists in quantitatively determining the potency or drug power upon healthy living tissues and adjusting the product assayed according to a fixed and definite standard of strength.

(3) Development

Pharmacodynamics is one of the most recent developments of medical science, it being a product of the last half century and more particularly of the last quarter. *Standardization* by pharma-

codynamic methods is a product of the last quarter century and more particularly of the last fifteen years.

Since pharmacodynamic or biologic standardization was first proposed, its importance has become more and more apparent, until to-day many methods are compulsory in the U.S.P. and N.F. and the subject is being taught in the leading colleges of Pharmacy and Medicine.

(4) History

The history of standardization may well be divided into six important steps. The *first step* was made by Dr. Lyman Spalding of New York City, who in 1817 submitted to the Medical Society of the County of New York the project for the formation of a National Pharmacopeia. His plan provided that the United States be divided into four districts, in each of which a convention was to be called composed of delegates from all the medical societies, schools, etc., in that district. Each district convention was to form a pharmacopeia and appoint delegates to a general convention in Washington. To this general convention the four district Pharmacopeias were to be taken and from the material thus brought together a National Pharmacopeia was to be compiled.

The adoption of Dr. Spalding's project resulted in the publication of the first National Pharmacopeia on Dec. 15, 1820.

The *second step* toward standardization was the formation and organization of the American Pharmaceutical Association in 1853, the object of which was to improve and regulate the drug market. The necessity for such a step was due to the importation of inferior, adulterated and deteriorated drugs and to the adulteration and sophistication constantly being practised in the United States at the time.

The *third important step* consisted in the adoption of the Purity Rubric and of assay processes for galenical preparations by the Pharmacopeial Convention of 1890.

The *fourth important step* consisted in the securing of legislation known as the Pure Food and Drugs Act of June 30, 1906, by which the standards of the Pharmacopeia were made Law for Interstate Commerce in drugs and medicines.

The *fifth important step* was made by the Pharmacopeial Convention of 1910 in recommending that the Revision Committee adopt *pharmacodynamic* methods for standardizing certain preparations of drugs not amenable to chemical standardization. This resulted in the inclusion of several optional and two compulsory biologic assay methods (Cannabis and Pituitary) in the U.S.P. IX.

The *sixth important step* was the inclusion of compulsory biologic assay methods in the U.S.P. *X* for nine drugs and their preparations.

(5) Drugs Requiring Biological Assay

The list of drugs to which it is necessary to apply biologic assay methods is not long, since all chemicals are standardized by means of chemical assay. The chemical method of assay is also used for such drugs as opium, belladonna, nux vomica, etc., in which the active constituents are capable of isolation in the *pure form.* There are, however, a number of drugs and their preparations which *cannot* be satisfactorily assayed by chemical methods, either for the reason that their active principles are *not known* or that they *cannot be quantitatively isolated in the pure state* by any of the known chemical methods. In this list are found such important drugs in common use as *aconitine, aconite, apocynum, cactus grandaflorus, cannabis, convallaria, digitalis, epinephrine, ephedra and ephedrine, ergot, gelsemium, piscidia erythrina, squill, strophanthus, and their preparations.* This list also includes many of the glandular products such as *anterior pituitary, corpus luteum, liver substance, posterior pituitary, ovarian residue, whole ovary, and the solutions of the extracts of these glands intended for direct medication.* Since there are no satisfactory chemical methods of assay for this list of drugs, recourse must be had to standardization by *pharmacodynamic* means.

(6) Variation in Strength of Non-standardized Preparations

Variability in the strength of crude drugs has long been a matter of common knowledge; a greater variability in the pharmacodynamic power and therapeutic usefulness of their preparations follows as an inevitable corollary.

Edmunds and Hale quote Fränkel as having reported a variation of from 100 to 275 per cent. in the strengths of infusions of digitalis and 100 to 400 per cent. in the strengths of tinctures of digitalis obtained by him in and around Heidelberg. The following table shows the variation in physiologic activity *before standardization* of some U.S.P. preparations assayed during one year in the author's laboratories.

TABLE I

Drugs	Number assayed	Variation, per cent.
Digitalis, Tr.	51	30 to 444
Digitalis, F.E.	16	26 to 160
Digitalis, S.E.	7	29 to 100
Ergot, F.E.	17	0 to 310
Aconite leaves, Tr.	6	38 to 111
Aconite root, Tr.	12	33 to 363
Aconite root, F.E.	7	52 to 266
Aconite root, S. E.	10	6 to 166
Aconite root, P.E.	11	5 to 100
Cannabis Indica, F.E.	15	40 to 150
Cannabis Indica, S.E.	4	71 to 125
Gelsemium, Tr.	7	64 to 156
Gelsemium, F.E.	7	65 to 220
Gelsemium, P.E.	3	88 to 187
Strophanthus, Tr.	12	55 to 277
Squills, F.E.	13	71 to 153

The above table shows that these *unstandardized* preparations ranged in strength from 0 to 444 per cent. It can be readily understood that with such a variation in the commercial preparations, the physician *cannot*, unless he employs "standardized" preparations, depend upon obtaining a definite effect as the result of a given dosage. If, for example, a druggist who *does not* dispense standardized preparations, fills a physician's prescription, calling for tincture digitalis, with a preparation corresponding in strength to the preparation in the above table which possessed only 30 per cent. of the standard activity, the doctor having failed to obtain the desired effect doubles the dosage. In the meantime, the druggist having replenished his "stock bottle," fills the new prescription with a

preparation corresponding in strength to the preparation in the above table possessing 444 per cent. of the standard activity. As a result of this, the patient instead of receiving a dose possessing, theoretically, twice the activity, receives one possessing 28 times the activity of the dose first prescribed.

(7) Necessity for Biologic Assay Methods

The above table also illustrates the great necessity for standardization and emphasizes the fact that it is not only essential to know that a drug contains valuable medicinal properties, but that *in order to secure the best therapeutic results these must be present in the commercial preparations in definite and constant amounts.* This can be obtained only by means of *standardization.* It is for this reason that the U.S.P. and N.F. now include compulsory biologic assay standards.

(8) Standardization of Crude Drugs

Standardization of crude drugs is in most cases not necessary since, as a rule, crude drugs are not administered as such and also because *the use of standardized crude drugs in the manufacture of galenical preparations does not insure uniformity in the finished product.* It is only necessary to *assay* the crude drugs in order to avoid the use of those which are inert or adulterated. Crude drugs, however, which are to be administered as such *must* conform to the U.S.P. or N.F. standards. Also the manufacturer of standardized preparations finds it necessary to *assay* his drugs before purchasing, since the value of the drug to him is in direct proportion to the amount of activity it possesses. If the manufacturer neglected this precaution, he would soon find himself out of pocket.

"Standardized" fluid extracts or tinctures do not necessarily result from the percolation of "standardized" drugs for the following reasons:

First, there is the personal equation of the pharmacist.

Second, though extracted under identical conditions, the resulting preparations may differ in strength.

Third, the variation in the amount of the active principles extracted from the same drug by ethereal or chloroformic solvents

and the amount extracted by alcoholic or hydroalcoholic solvents—the therapeutic value of the crude drug is determined by extracting the active principles with ethereal or chloroformic solvents and therefore is not necessarily the same as that of the preparation made from it by percolation with an alcoholic or hydroalcoholic menstruum.

Fourth, the decomposition of some alkaloids produced by the heat employed in concentrating the percolate to a fluid or solid extract.

Aconite is a notable example of number five. The aconitine when subjected to the heat of concentration is often partly split up into its decomposition products—aconine and benz-aconine—which are practically physiologically inert. Due to this fact it often occurs that a solid or powdered extract made from a very potent drug is almost inactive. For this same reason the chemical assay for powdered and solid extracts of aconite is practically valueless. This assay is based upon the total alkaloidal content, which is determined by titrating the alkaloidal residue with a standard acid solution, making no distinction between the relative amounts of aconitine, aconine and benzaconine present. It can readily be seen that by this method the inert aconine and benz-aconine will neutralize the standard acid solution and thus give the same percentage results as would be obtained if the residue consisted of active aconitine alone.

It is therefore impossible to prepare standardized fluid extracts, tinctures, etc., from assayed drugs without assaying and adjusting the *finished* products.

(9) Deterioration

One of the problems of standardization is to prevent finished products from deterioration. Physicians ask, "of what value is standardization, if the carefully assayed and adjusted preparations, due to deterioration, lose part or all of their activity before being placed in our hands for use?" Fortunately, only a few drugs and their preparations are subject to rapid deterioration. Most of the drugs, such as nux vomica, belladonna, cinchona, opium, etc., have definite alkaloids as their active principles. Preparations of these

drugs are quite stable and will, if properly kept, maintain their activity for years.

On the other hand, digitalis, aconite, and ergot preparations, *particularly ergot*, deteriorate quite rapidly when exposed to the ordinary conditions under which galenical preparations are kept. In the case of these drugs the activity is usually greatly impaired, in many instances, in from two to three months.

The subject of deterioration of these drugs has for a long time occupied the minds and attention of various workers but until recently no one had succeeded in determining a method by which manufacturers of preparations of these drugs could put them on the market in a form in which they would remain stable for a definite time.

The principal causes of such deterioration are oxygen of the air, and heat. It is a known fact that fluid extract of ergot retains its activity for a much longer period of time when kept in well-filled and tightly stoppered containers.

To determine the value of a complete exclusion of air, a series of experiments were undertaken by Pittenger and Vanderkleed[1] with fluid extract of ergot.

First of all, this extract was tested by intravenous injections of 0.08 c.c. per kilo into dogs, and gave an immediate rise of blood-pressure represented by 44.8 mm. of mercury. The assay for total alkaloids by the process of Keller gave a percentage of 0.163. This fluid extract of ergot was then divided into four portions, as follows:

A—The first portion was put up in vacuum, in tubes specially designed and made for this purpose.

B—The second portion was filled into bottles which were tightly corked, and allowed to remain for one year unopened.

C—The third portion was filled into bottles which were kept loosely corked for one year, this being obtained by boring a small hole in the cork.

D—The fourth portion was tightly corked but opened occasionally throughout the year.

These four samples were tested upon dogs at the end of twelve months, with the result that with *A*, no loss of blood-pressure raising

[1] "A New and Reliable Method for the Preservation of Ergot Preparations," by Pittenger and Vanderkleed, Jour. A. Ph. A., Aug., 1912, p. 799.

power was sustained; this was also true of the percentage of total alkaloids. Of the other samples, that tightly corked (*B*) deteriorated the least (about 35 per cent.), but the strength of all except *A* was less than at the beginning of the experiments. A marked deterioration was noted in *C* and *D*, *D* (tightly corked but opened occasionally, conditions under which the preparation is commonly kept), showing the most marked deterioration (67 per cent.).

These investigations proved that by adopting the *vacuum method* (complete exhaustion and exclusion of the air from both the container and its contents and then hermetically sealing the container under vacuum) the rate of deterioration can so be retarded as to make these preparations of stable quality for at least a definite time, thus making it possible not only to supply the physician with preparations of these drugs, which will maintain their "standard strength," but also afford the experimenter a means of preserving standard preparations with which he can compare the strengths of new lots of drugs or their preparations and thus adjust them to standard strength.

Experience has shown that the most rapid deterioration, in most galenical preparations, takes place during the first three to four months. Some preparations which deteriorate rapidly during this period are comparatively permanent thereafter. This is the reason for the U.S.P. requirement that the standard fluid extract of ergot, supplied by the Bureau of Chemistry, be aged six months before standardizing.

The author has also proven that practically all digitalis preparations deteriorate the most during the first three months after manufacture.[1,2]

In the summary of the results of one of these experiments ([2]) the following statement appears:

"The tabulated results show the average deterioration in 9 to 13 months of 43 samples to be 18.8 per cent. The average deterioration of 38 samples during the first three or four months being 4 per cent. per month while the average deterio-

[1] The deterioration of "U.S.P." and "Fat-Free" Tinctures of Digitalis, by Paul S. Pittenger and H. K. Mulford, Jr., Journ. A. Ph. A., March 1918, 236.

[2] The Deterioration of Tincture of Digitalis by Paul S. Pittenger, Journ. A. Ph. A., December 1918, 1031.

ration of 32 samples after the first three or four months was 2.4 per cent. per month."

(10) Type methods

Many methods used for the physiologic standardization of drugs are not new but merely *quantitative* applications of methods used to elucidate the drug's physiologic action. The principal task of the biologic chemist, therefore, is the selecting of the most suitable method for the particular drug under consideration.

The principal type methods available for the physiologic study and standardization of the vegetable drugs are the three following:

1. *Toxic methods* in which guinea-pigs, frogs, or some of the higher animals are used, the value of the drug or preparation depending upon the amount required to cause the death of the animal. Examples: *a*, Reed and Vanderkleed's Guinea-pig Method; *b*, the one-hour frog method; (*c*), the twelve-hour frog method; and *d*, Hatcher's cat method for the standardization of the heart tonics and depressants.

2. *The amount of drug or preparation required to produce some specific effect upon the intact animal.* Examples: *a*, Cock's comb method for ergot; *b*, the blood-pressure method for epinephrine, pituitary extract, ergot, digitalis, etc.; *c*, the uterine method for ergot.

3. *The amount required to produce a definite effect on an isolated organ.* Examples: *a*, isolated uterus method for ergot and pituitary extracts; *b*, the perfusion method for the digitalis series.

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CHAPTER II

ACTION OF THE CARDIAC STIMULANTS AND DEPRESSANTS

The most important drugs which require biologic assay included under the above heads are:

(a) Stimulants
- Apocynum.
- Cactus grandiflorus
- Convallaria.
- Digitalis.
- Squills.
- Strophanthus.
- Epinephrine.[1]

(b) Depressants
- Aconite.
- Gelsemium.
- Veratrum.

Stimulants.— The cardiac stimulants are commonly known and referred to as the "*digitalis group*" because of the similarity of their actions to those of digitalis.

The distinguishing feature of this group consists in its power to produce an increased tone of muscular tissue, generally manifested most conspicuously on arterial and cardiac muscle, leading to increased strength and duration of the systole, and to rise of blood-pressure.

This series of drugs possesses a local and a general action. The action on the heart, however, is the most important of all, and is what distinguishes digitalis and its allies from all other substances. This action has been studied most carefully in the frog, and is found to be due to an alteration in the cardiac muscular tissue. On expos-

[1] The actions and standardization of epinephrine are treated separately in Chapter V, p. 61.

ing the frog's heart and watching its movements after the injection of digitalis, the muscular action can generally be made out very distinctly (Fig. 1). The heart becomes slower in rhythm, and contracts to smaller dimensions in systole, while it does not dilate so fully in diastole. During systole it is, therefore, whiter than in normal contraction, while during diastole it is less purple than in normal dilatation, owing to its containing less blood at each period. The

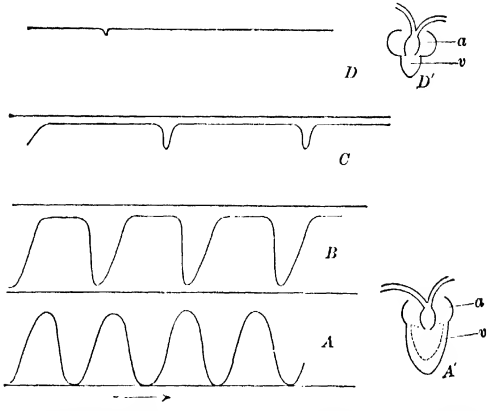

FIG. 1.—Tracing of the movement of the frog's ventricle under digitalis. The lever forms an upward stroke during systole. *A*, normal. *B*, the systole is somewhat more complete and is very prolonged, and the rhythm is correspondingly slow. *C*, the ventricle remains in systole with occasional feeble diastolic movements. *D*, the diastoles of the heart have almost entirely ceased. *A'*, diagram of the heart of the frog in its normal dimensions, *a*, auricle; *v*, ventricle with the aortic bulb rising from it. The dotted line in the ventricle represents the outline in systole, the continuous line the outline in diastole. *D'*, outline of the heart in standstill after digitalis. The ventricle *v* is very much contracted, the auricle *a* distended with blood. (Cushny.)

slowing can be seen to be due to the heart's remaining contracted longer than usual, while the dilatation is very short and imperfect. Later the apex of the ventricle ceases to dilate during diastole, and remains quite still while the base still dilates after each auricular systole. Or the whole ventricle dilates only once for every two contractions of the auricle, or the two halves of the ventricle may contract alternately so that the blood is thrown from one side to

the other. Meanwhile the duration of systole becomes still more prolonged, and the extent of diastolic dilatation diminishes until the ventricle finally ceases to relax, remaining in a position of extreme systole with its cavity obliterated. The auricles come to a standstill also, but they are unable to empty themselves into the contracted ventricle and therefore remain distended with blood. The typical action of digitalis on the muscle of the frog's heart, then, consists in a tendency to increase and prolong contraction, and diminish and shorten diastole (Cushny).

The action of the digitalis series may be divided into three stages:

1. The *therapeutic or first stage* characterized by an acceleration of the heart and a rise in blood-pressure.

2. The *inhibitory or second stage* causing a low blood-pressure from a lessened output of the heart.

3. The *toxic or third stage* characterized by marked irregularities of the heart, during which the blood-pressure rises again from the increased output of the heart and the further contraction of the vessels.

Method of Recording the Action of the Heart Tonics and Depressants (and Other Drugs) upon the Frog's Heart

Apparatus Necessary for Experiment.—Small kymograph, iron stand with clamps, frog board, Harvard heart lever; probe, scissors, tweezers, scalpel, pipette, normal salt solution; drug solutions. If a time tracing is desired the experiment will also require a signal magnet, dry cells and an electric clock.

Animals.—Medium size frogs of about 35-40 gm. are best adapted for this purpose.

Preparation for Experiment.—The drum is removed from the kymograph, covered with glazed paper, smoked and replaced. Next the frog board, heart lever, and signal magnet are assembled as shown in Fig. 2. The frog is then *pithed.* This is accomplished as follows:

Wrap the frog in a towel allowing only the head to protrude. The frog is held in the left hand and the head bent slightly forward with

the left thumb. If the nail of the right forefinger is passed lightly along the spine the articulation between the skull and the vertebral column can be felt at the point where the cerebrospinal canal has no bony covering. The canal is punctured at this point by a narrow-bladed knife after which the brain is destroyed by inserting a probe at this point and pushing it into the brain cavity, gently moving

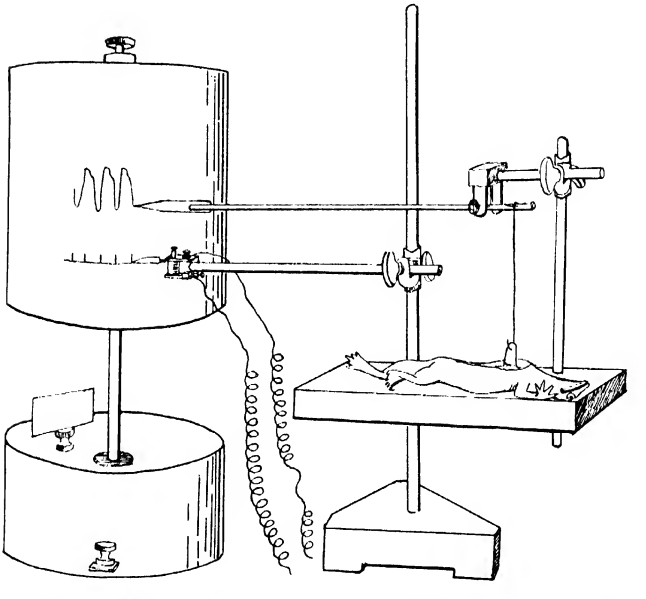

FIG. 2.—Showing arrangement of apparatus for recording the action of the frog's heart in place in the body. (From Greene's Experimental Pharmacology.)

it from side to side. The point of the probe is now turned and the spinal cord is destroyed in a similar manner. This final stimulation of the nerve cells causes a discharge of motor impulses to the muscles of the body, which give a series of convulsive twitches or contractions. These twitches quickly cease, the body and limbs become toneless and relaxed and the reflexes abolished.

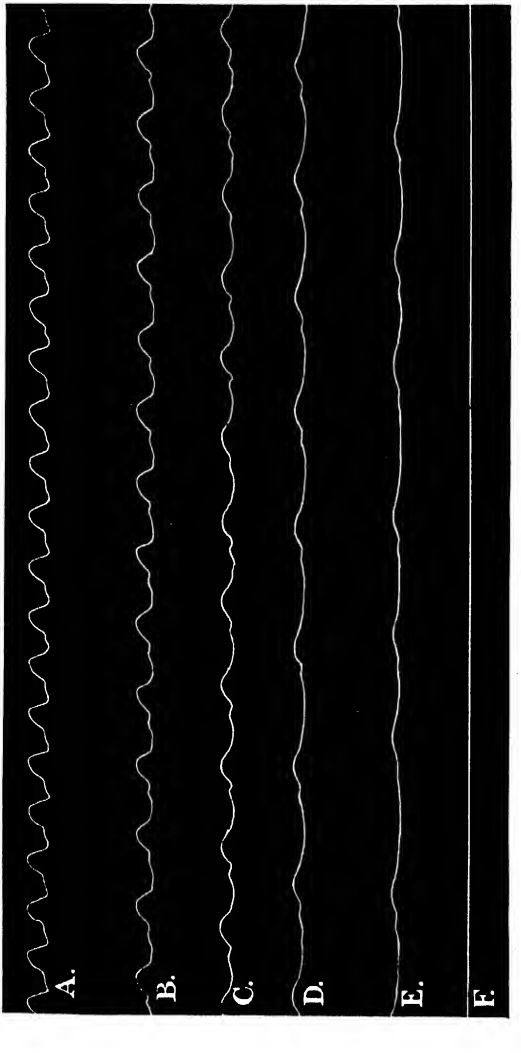

FIG. 3.—Action of digitalis on frog's heart. The lever forms an upward stroke during systole. *A*, normal; *B*, *C*, *D* and *E* show the action of the drug. Note the gradual lengthening of the systole, also the gradual decrease in the amplitued of the contractions caused by the ventricle relaxing less and less during diastole; *F*, heart tonically contracted in systolic standstill.

The animal is pinned on a frog board with the abdomen uppermost; the skin over the abdomen is pinched up and slit to the mouth; the abdominal wall is then divided slightly at one side of the middle line to avoid cutting the anterior vein of the abdomen. By a transverse cut the sternum is then divided; the junction of the anterior abdominal vein with the heart is preserved. The chest girdle is next divided in the middle line, the inner blade of the scissors being kept hard against the sternum to avoid injuring the heart beneath. The divided halves of the pectoral girdle are then pulled widely apart and the heart is found to be enveloped by a thin membrane, the pericardium. Pick up the pericardium with the forceps and slit it open. On the posterior surface of the heart is a slender band of connective tissue, the frænum, which should be divided. Next hook a small sized pin into the tip of the heart, attach to it a thread which is next to be connected with the heart lever. Counterpoise the heart lever and let it record the movements of the heart on the smoked drum.

Preparation of Solutions.—Normal saline solution for frogs is prepared by dissolving 30 gm. of sodium chloride in 4000 c.c. of distilled water. The *drug solution* is prepared by dissolving the drug in physiological saline. If the preparation to be tested is a tincture or a fluid extract it should be freed from the greater part of alcohol by evaporation on the water bath, and diluted with normal saline solution to the desired volume.

Experiment. *Effect of the Heart Tonics on the Frog's Heart.*—First record the normal movements of the heart, then irrigate with solution of digitalis for about two minutes. Take several tracings one beneath the other until the heart finally comes to standstill in systole. (See Fig. 3.) The heart is slowed by lengthening of the systolic contractions, which are also more powerful. At first the output of the heart is also increased. As the action progresses the systole becomes longer and stronger and the ventricle relaxes less and less during diastole, retaining a remarkably white appearance. The output is lessened, diastolic relaxation is finally abolished altogether, and the heart remains tonically contracted, in systolic standstill.

"In some cases certain other features appear in the frog's heart, for the slow rhythm may be accompanied by a less perfect systole,

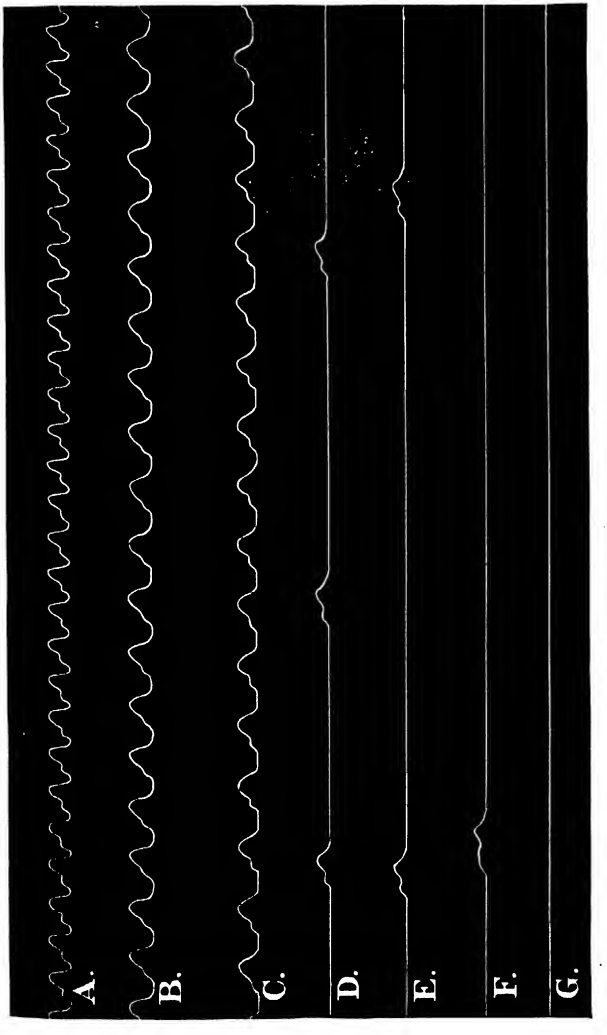

FIG. 4.—Action of aconite on frog's heart. *A*, normal; *B*, *C*, *D*, *E*, *F*, and *G* show the slowing and final stoppage of the heart due to the action of the drug.

and instead of the heart ceasing in systole it may come to a temporary standstill in a state of extreme diastolic dilatation. This is due to stimulation of the vagus center in the medulla, and must be carefully distinguished from the action on the cardiac muscle. Not infrequently the two forms occur in combination, or the symptoms of inhibitory actions precede those of the true cardiac change" (Cushny).

Depressants.—The principal action of the members of this group is a lowering of the activity of the heart. There are two ways in which the drug accomplishes this, *i.e.*, 1. by stimulation of the vagus mechanism; 2. by weakening of the cardiac muscle itself. The latter effect may be produced, however, by large doses of almost any drug; therefore, the former is alone useful therapeutically.

Experiment. *Effect of the Depressants upon the Frog's Heart.*—Arrange apparatus as described under "Heart Stimulants" (page 15); connect heart with writing lever and record normal movements; irrigate with 0.1 per cent. solution of aconite in Ringer's solution for about two minutes. Take several tracings one beneath the other until the heart stops. (See Fig. 4.)

It will be noted by Fig. 4 that the frog's heart after preliminary quickening from stimulation of the accelerator endings and cardiac muscle soon passes into a state characterized by slow pulse and prolonged diastole, produced by stimulation of the inhibitory mechanism.

Effect of Heart Tonics and Depressants upon Blood-pressure. *Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Method of Injecting.*—Same as required for the standardization of epinephrine. (See page 64.)

Experiment. *Effect of Heart Tonics.*—After all preliminary arrangements have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood-pressure tracing is then started on a slowly revolving drum. After obtaining a tracing of normal pressure about three inches in length, inject a toxic dose of tincture of digitalis into the saphenous vein; take continuous tracing until heart stops. The best tracings are produced by doses which cause the stoppage of the heart in about fifteen to twenty minutes. (See Fig. 5.)

"In the *first or therapeutic stage* of the action of this series, the rhythm of the heart is changed and the extent of contraction and relaxation of the ventricle and auricle undergo certain modifications. The rhythm of the heart is distinctly slower than before giving the drug, for the inhibitory apparatus is set in activity and the slowing is accordingly due to a prolongation of the pause in diastole. The ventricles contract to a smaller size, that is, they empty themselves much more completely than they normally do. It is now universally recognized that the normal ventricle does not empty itself completely; that even at the end of its systole there still remains some blood in its interior. After the action of this group has begun, however, the blood remaining at the end of systole is much less than before. This increased contraction is, like that in the frog's heart, due to action on the cardiac muscle. The papillary muscles undergo the same changes as the rest of the ventricular wall, contracting more strongly and more completely than before the administration of the drug.

"In the *second stage* the symptoms are due to excessive inhibitory activity, while the direct cardiac action is less developed. The rhythm of the ventricle, and consequently of the pulse, is very slow

FIG. 5.—Effect of digitalis on blood-pressure. Drug injected intravenously from *X* to *X*; *A*, normal; *B*, fall in pressure during the injection, caused by the local irritant action on the heart and vessels; *C*, therapeutic stage; *D*, toxic stage.

and irregular, as is always the case when the inhibitory apparatus is strongly stimulated. During diastole the ventricle dilates more completely than usual, while its systole varies in strength. If the muscular action is well developed, it continues to empty itself more completely than usual, but very often the inhibition is so powerful that the muscular action is entirely concealed and the systole is weaker and more blood remains at the end of the contraction than

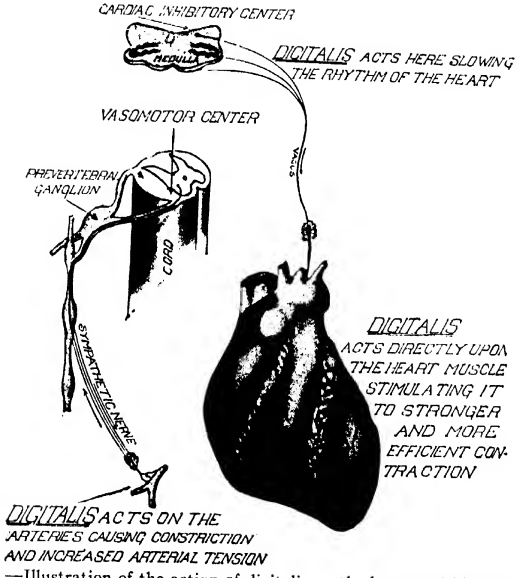

FIG. 6.—Illustration of the action of digitalis on the heart and blood-vessels.

before the drug was administered. As a general rule, however, each beat expels more blood than normally, because the heart is engorged before the systole begins; but the rhythm is now so slow that the output per minute and the efficiency of the heart as a pump is less than usual. This is the feature which differentiates the first from the second stage, in which the same factors are present; in the first stage the efficiency of the heart, *i.e.*, the amount of blood

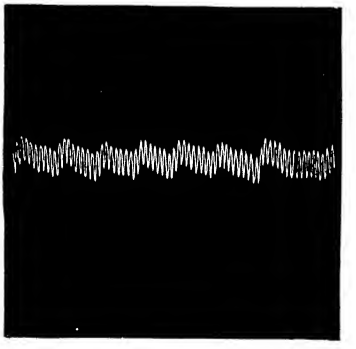

FIG. 7.—Normal blood-pressure tracing. The upward stroke represents the heart systole, down-stroke the commencement of diastole.

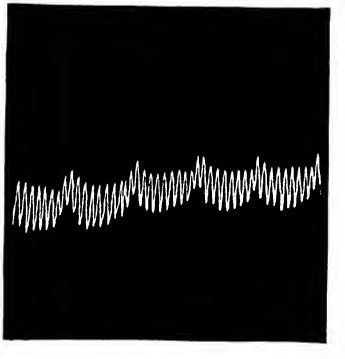

FIG. 8.—Action of digitalis, therapeutic stage. Note the increased length of up-stroke (increased strength of contraction); also the increased space between the up-strokes (prolonged diastole).

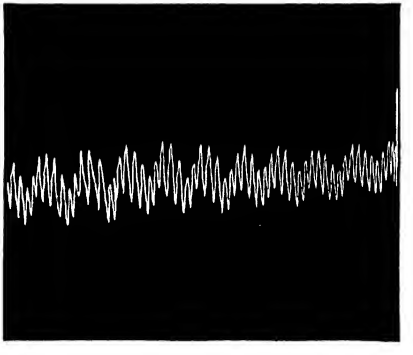

FIG. 9.—Action of digitalis. Combination of therapeutic stage with beginning of intermediate stage; characterized by slight irregularity and exaggerated inhibition from action of the drug upon the inhibitory center in the medulla. Note irregular length and increased space between strokes, showing irregularity of the heart's action preceding the toxic stage.

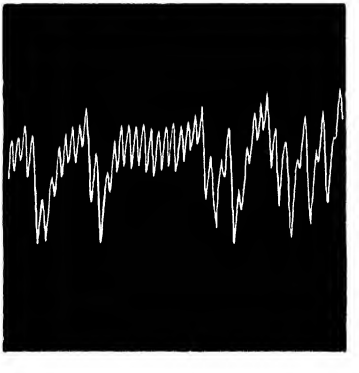

FIG. 10.—Action of digitalis. Extreme inhibition and irregularity.

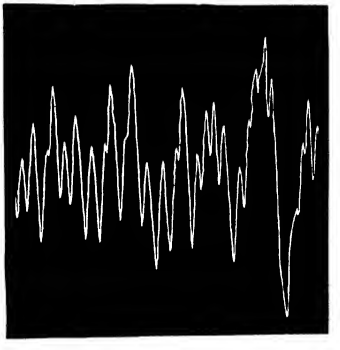

FIG. 11.—Action of digitalis. Marked toxic action.

FIG. 12.—Action of digitalis: toxic stage. Delirium cordis, preceding the death of the animal about five minutes. Note the weakness of the contractions and extreme irregularity.

expelled per minute, is greater, in the second stage less than before the administration of the drug.

"When very large quantities of any of this series are injected, the *third stage* sets in. It is preceded by the first for a short time, generally by both first and second. In this stage the ventricular rhythm becomes very much accelerated, often beyond the normal, and even beyond that seen after paralysis of the inhibitory nerves. This acceleration has often been supposed to be produced by paralysis of the vagus, but this is not the correct explanation, for stimulation of this nerve sometimes still slows the heart and all this causes dilatation. The acceleration is really due to the drug increasing the irritability of the heart muscle to such an extent that the inhibitory apparatus is no longer able to hold it in check. All the features of the third stage are due to the poison's increasing the irritability of the heart muscle. This leads to acceleration of the beat, and, eventually, through the muscle of one pair of chambers being acted on more than that of the other, to arrhythmia. The extra-systoles are evidently of the same origin, and the final delirium is also to be ascribed to this action" (Cushny).

Figures 7, 8, 9, 10, 11 and 12 demonstrate the various stages of digitalis action on the blood-pressure.

Experiment. *Effect of Depressants.*—Follow directions given under experiment with heart tonics on page 20, substituting tincture of aconite for tincture of digitalis.

Figure 13 shows the effects of a toxic dose of aconite upon blood-pressure. It will be noted that large doses besides stimulating the vagus also exert a direct muscular action and thereby greatly increase the force and especially the rate of the heart, at the same time rendering it extremely arrythmic. As the direct muscular action comes into play the heart suddenly accelerates from the slow vagus rhythm to one far above normal. Irregularities follow in many different forms after which the heart finally goes quite suddenly into delirium cordis, and stops. The marked acceleration of the heart is due in part to the paralysis of the inhibitory apparatus, but mainly to the direct action on the cardiac muscle. There is, however, no reason to suppose that the direct cardiac action comes into play from the administration of therapeutic doses of the drug.

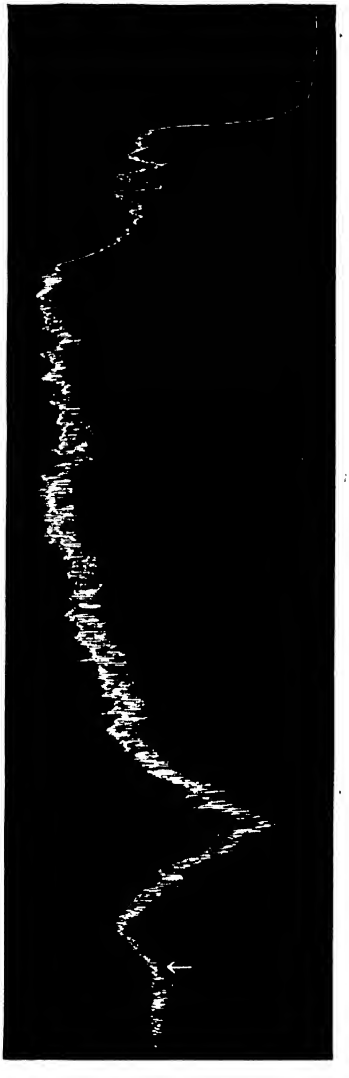

FIG. 13.—Effect of aconite on blood-pressure. Drug injected at arrow. See text.

That the acceleration is mainly due to stimulation of the cardiac muscle itself is proven by the fact that the quickening occurs even in the nerve-free heart of the embryonal chick, also by the fact that the drug produces acceleration of the mammalian heart after the nerve endings have previously been paralyzed.

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CHAPTER III

STANDARDIZATION OF CARDIAC STIMULANTS

Several methods are available for the quantitative determination of the activity of these drugs and of their preparations. The three principal type methods being:

1. A toxic method, in which guinea-pigs, frogs, goldfish, and some of the higher animals are used, *e.g.*, Reed and Vanderkleed's guinea-pig method; Hatcher's cat method; The Official U.S.P. and N.F. "one-hour" frog method; Houghton's "twelve-hour" frog method, Focke's frog method and Pittenger's "gold fish" method.

2. The effect upon blood-pressure.

3. The effect upon the isolated heart of the frog or turtle. (*Example:* Perfusion method.)

1. TOXIC METHODS. (a) **Frogs 1. The Official "One Hour" Method.**—This method consists in determining the minimum dose of the drug, per gram body weight of animal, that will cause permanent systole of the frogs ventricle at the end of exactly one hour. It is the official method of the U.S.P. and N.F. for the standardization of the cardiac stimulants,—Digitalis, Strophanthus, Squill and the official preparations of these drugs.

Apparatus Necessary for Experiment.—One all-glass syringe or sharp-pointed pipette, graduated into hundredths of a cubic centimeter; frog-boards; tweezers, scissors, scalpel, probe; tank arranged for maintaining constant temperature, equipped with individual numbered frog cages; balance for weighing frogs, analytical balance and volumetric flasks for making dilutions.

Animals.—Use healthy frogs of the same species (*Rana pipiens* Schreber) commonly known as "grass" or "leopard" frogs. This is very important as it is impossible to obtain satisfactory results if frogs of different species are used. The commonly known "bull frog" is not nearly as satisfactory.

Since the dose is computed per gram of body-weight, frogs of any size may be used, but it is thought best to use only those of medium weight (20 to 30 gm.) and of nearly uniform size, varying from one another not more than 5 gm.

Care of Animals. -See p. 244.

Preparation of Experiment.—Another precaution in using frogs as assay animals is that the experiments should be carried out at the same temperature on account of the easy susceptibility of the frog to heat. A temperature of about 20° C. is preferable, because, being about ordinary room temperature, it can easily be maintained. This may best be accomplished at all seasons by means of a simple apparatus consisting of a large galvanized iron tank partly filled with water in which are placed the small cages containing the frogs. The temperature of the tank being raised or lowered merely by heating or cooling the water. (See Chapter XIV "Temperature Regulation" page 199.)

The day before the frogs are to be used, a sufficient number should be taken from the storage tanks and placed in a tank, the temperature of which is approximately 20° C. One hour before the assay, they are weighed to within 0.5 gm. and placed in individual numbered wire cages or containers in a tank containing water to the depth of about 1 cm. (1/2 inch), the water being kept at uniform temperature of 20° C. during the assay.

Preparation of Solutions.—Crude drugs cannot be administered to the frogs as such. The U.S.P. and N.F. therefore direct that a tincture or fluidextract be prepared from the drug and the liquid preparation tested according to the method specified.

Powdered or solid extracts, tablets, etc., should be diluted to tincture or fluidextract strength. In all cases care must be taken that the solvent used is of the same alcoholic strength as that used in the original extraction of the drug. This is necessary, in many cases, to effect complete solution of the active constituents.

The formula used in the preparation of the solid substance should be consulted and the diluent prepared in accordance with the alcoholic content of the original menstruum.

The preparation to be tested is diluted with a sufficient quantity of distilled water to make the doses injected as uniform in quantity

as possible, approximately 0.015 c.c. for each gm. body weight of frog. In case the alcoholic content of any preparation after dilution is higher than 20 per cent. the preparation may be subjected to careful evaporation and subsequent addition of distilled water until the original volume is restored and the alcohol content is not above the per cent. named.

Method of Injecting.—The solution to be tested is accurately measured by means of an all-glass syringe or sharp-pointed pipette. The floor of the mouth under the tongue is then punctured and the contents of the syringe or pipette delivered directly into the anterior lymph-sac of the animal, *care being taken not to puncture the skin.* (See Fig. 129.) The skin of the cold blooded frog is not nearly as elastic as that of warm blooded mammals. When the frog's skin is punctured with the syringe needle the hole does not close when the needle is withdrawn and part of the injected preparation oozes through the opening and is lost. Therefore, if when inserting the needle, the skin should be accidently punctured the frog must either be discarded or the injection given under the skin of the back, after first passing the needle through the leg muscle.

Standard Preparation.—The use of a standard is made necessary by the fact that frogs differ at different seasons of the year, in their susceptibility to the members of the digitalis series. The condition of susceptibility is ascertained and allowed for by the use of a solution of *Ouabain* as a standard.

The ouabain employed must be equal in activity to the "standard" ouabain supplied by the Bureau of Chemistry, U. S. Department of Agriculture.

Aqueous solutions of ouabain are not stable. Solutions in 95 per cent. alcohol are practically permanent. It is very convenient, therefore, in routine laboratory work to prepare the standard ouabain solution as follows: Very accurately prepare about 12 c.c. of a 1 in 1000 solution of ouabain in 95 per cent. alcohol. Place this solution into ten insoluble glass ampuls containing 1.2 c.c. each and carefully seal. This ampul solution will remain permanent for several months.

From the ampul solution prepare a second solution of the strength of 1 in 10,000 by adding 1 c.c. of the alcoholic ampul solution to 9

c.c. of distilled water; this latter solution should be *freshly prepared* for each assay.

Actual Standardization.—Remove from the constant temperature bath a series of four healthy, previously weighed frogs of standard weight (20–30 gms.) which have been kept at constant temperature as described under "Preparation of Experiment." Inject them with 9/10, 10/10, 11/10, and 12/10, respectively, of the standard dose of the preparation being tested for each gram body-weight of animal. The doses should be calculated and measured to the hundredth of a c.c. The animals are replaced in their respective cages in the "constant temperature" tank after injection, the temperature being maintained at 20° C.

About fifty-eight minutes from the time of injection, each frog is *pithed* (see p. 15), the heart exposed, and its condition examined. If this is already completely paralyzed, the dose has been excessive; if the pulsation still continues, although at a diminished rate, the dose has been insufficient. For the correct end reaction, at the expiration of one hour from the time of injection, the ventricle must be in systolic standstill, while the auricles are widely dilated. Following mechanical stimulations, feeble contractions may occur in the auricles and localized contractions in the ventricle, but no general contraction is allowable.

If, when the lymph sac is opened to expose the heart, any of the injected drug is found unabsorbed, the animal must be discarded and not considered in the results obtained.

It happens sometimes that one frog out of a large series may prove a decided exception to the others in the way of increased or decreased susceptibility to the drug. Such an animal should also be discarded.

After the preliminary or trial assay has been carried out and the approximate strength of the preparation ascertained, a second series of frogs are injected in like manner, using doses, the limits of which are considerably narrower than the first series. A third or even fourth series of injections may be necessary to confirm the earlier results.

In other words, the dose of the drug must be progressively increased or decreased, as the case may be, until the results show the *minimum* amount of the drug per gram body weight of frog, that

will cause permanent systole of the frog's ventricle in exactly one hour.

The dose thus found is then compared with the dose of the standard ouabain, which is similarly ascertained upon another series of frogs of the same lot. From such a comparison the strength of the unknown preparation can be suitably adjusted.

The necessary strength of an unknown preparation may be calculated from the dose actually found by the ratio of the standard dose of ouabain to the dose of ouabain found necessary to kill. Thus:

Standard dose of ouabain	:	Found dose of ouabain	::	Standard dose of drug being assayed	:	Necessary dose of unknown

The standard dose of ouabain is 0.00,000,05 gm. per gm. of body weight of frogs.

If, therefore, in a certain series of frogs the resistance to ouabain is found to be increased so that a dose of 0.00,000,075 gm. is necessary to stop the heart in systole within one hour, the dose of the tincture of digitalis would also have to be increased to 0.009 c.c. and the actual dose found calculated against this, instead of against the standard dose of 0.006 c.c. For example, in frogs showing the increased resistance as indicated above, if the tincture being examined assays 0.018 c.c. per gm. of body weight of frog, it is one-half strength.

$$0.00,000,05 : 0.00,000,075 :: 0.006 : 0.018 = 50 \text{ per cent.}$$

Accuracy of Test.—Dr. J. C. Munch of the Bureau of Chemistry, U. S. Dept. of Agriculture recently directed a series of co-operative tests in order to determine the accuracy of this method. Three samples of tincture of digitalis labeled A, B and C respectively were forwarded to biologic assayists throughout the country for assay. The results of this investigation showed a variation of less than 10 per cent. in the assay results of experienced technicians. These results would tend to prove, therefore, that this method is accurate to within 10 per cent.

Official Standards.— The following are the official standards for the drugs and their preparations directed to be assayed by this method:

Digitalis U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 c.c. of the tincture, equivalent to 0.00,000,05 gm. of ouabain, for each gram of body weight of frog.

Tincture of Digitalis U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 c.c. and not more than 0.0065 c.c. equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gm. of body weight of frog.

Squill U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 c.c. of tincture, equivalent to 0.00,000,05 gm. of ouabain for each gm. of body weight of frog.

Fluidextract of Squill U.S.P., properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.00,055 c.c. and not more than 0.00,065 c.c. of fluid extract, or the equivalent in fluidextract of not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body weight of frog.

Tincture of Squill U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 c.c. and not more than 0.0065 c.c., equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body weight of frog.

Strophanthus U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0,00,006 c.c. of the tincture, equivalent to 0.00,000,05 gm. of ouabain, for each gram of body weight of frog.

Tincture of Strophanthus U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.00,005,5 c.c. and not more than 0.00,006,5 c.c., equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body-weight of frog.

Fluidextract, Digitalis, N.F. when assayed biologically the minimum lethal dose should be not greater than 0.0006 of the Fluidextract, or the equivalent in fluidextract of 0.00,000,05 gm. of ouabain, for each gm. of body weight of frog.

2. **Houghton's "Twelve-hour" Method.**—With this method the strength of the drug is determined by finding the minimum amount per gram body-weight necessary to cause the death of the animal within twelve hours.

Apparatus Necessary for Experiment; Animals; Care of Animals; Preparation of Experiment; Preparation of Solutions; Method of Injecting.—Same as given under "One Hour Method" p. 29.

Actual Standardization.—The general procedure of injecing several series of animals with progressively increasing or decreasing doses of the preparation to be tested is essentially the same as that given for the "one hour" frog method, differing only in the following points: 1. the results are recorded at the end of twelve hours instead of one hour; 2. the end reaction is the death of the animal instead of the stoppage of the heart.

A further development of this method of assay led to the adoption of a **"heart tonic unit"** (H.T.U.) as a means of expressing the physiologic values, the number of such units to be derived directly from the minimum lethal dose of the given preparation. A convenient unit is obtained by dividing one by the minimum lethal dose per gram body-weight of frog; or, in other words, the number of units will be the reciprocal of the minimum lethal dose. To illustrate; if the minimum lethal dose of a given drug per gram body-weight of frog is found to be equivalent to 0.01, then the given substance, assuming that it belongs to the group of heart tonics, would contain 100 heart tonic units; $\left(\text{M.L.D.}\frac{1}{0.01}\right) = 100\text{(H.T.U.)}$. This rule can be applied to any of the heart tonics as a means of expressing such values in whole numbers.

Instead of stating the full number of heart tonic units in all cases round numbers can be used, which do not vary more than a given per cent. from the actual number of units (Houghton).[1]

Standards.—This method is *not official* and there are, therefore, no official standards.

Experience has shown, however, that the figures given in the following list indicate what may be taken as tentative standards for the most important preparations of the digitalis series of heart tonics when assayed according to this method.

TABLE II

	M.L.D.	Exact No. of H.T.U. per c.c.	No. of H.T.U. in round numbers per c.c.
Digitalis:			
Fluid extract, U.S.P., 1890.....	0.0015	66	65
Solid extract.....	0.0005	200	200
Tincture, U.S.P., 1900.....	0.015	6	6
Digitalin (Germanic).....	0.00005	2000	2000
Squill:			
Fluid extract, U.S.P., 1890.....	0.0012	83	80
Strophanthus:			
Tincture, U.S.P., 1900.....	0.000075	1300	1300
Convallaria—Fluid extract:			
Rhizome and roots, U.S.P.....	0.00025	400	400
Herb.....	0.00015	666	650
Flowers.....	0.00009	1111	1100

On account of the variation in the toxicity of the standard and of the different preparations belonging to this series, due to changes in the resistance of frogs, it becomes a matter of considerable importance, as well as convenience to have a table to which one can refer and readily deduce the number of H.T.U. of any preparation after obtaining its M.L.D. A table very well adapted to this purpose is the one prepared by H. C. Hamilton[2] (pages 38 and 39). "The number of H.T.U. in any given preparation is the reciprocal

[1] E. M. Houghton: The Lancet, June, 19, 1909.

[2] H. C. Hamilton: Amer. Jour. Pharm., Feb., 1912.

of ten times the M.L.D. if the frogs are of normal resistance. The resistance of frogs, however, varies greatly, and for this reason the number of H.T.U. per cubic centimeter can evidently not be obtained in so simple a manner.

The factor to be used for adjusting its value is the ratio between the M.L.D. of the standard selected and its average M.L.D. The formula would, therefore, be

$$\frac{1}{10 \times \text{M.L.D. of sample}} \times \frac{\text{M.L.D. of standard}}{\text{Average M.L.D. of standard}} = \text{H.T.U. per c.c. or gm.}$$

By means of this formula the correctness of any number in the table may readily be verified.

In Table III the numbers in the first horizontal column are the M.L.D. of standard tincture strophanthus, U.S.P., 1890, the range of doses being such as to cover the variation in its toxicity to frogs during the different seasons of the year.

The eight horizontal columns of numbers following this are the M.L.D. for each preparation of the series, with the same range in toxicities as for the tincture mentioned first.

The numbers in the first vertical column beginning with 0.010 are M.L.D. of samples. In this column will be found every possible M.L.D. of members of this series by merely adjusting the decimal point.

All the other numbers in columns A to I inclusive and below the double line are H.T.U. per cubic centimeter or per gram of preparations of the digitalis series of heart tonics, any particular number being the value in terms of heart tonic units of a sample whose M.L.D. is at the head of the horizontal column and the M.L.D. of the standard is in the vertical column which intersects the horizontal at that number.

For example, if a tincture of digitalis has an M.L.D. of 0.02 while that of the standard tincture digitalis is 0.012, it contains 4 H.T.U. per cubic centimeter, this number being found where the columns headed D and 0.020 intersect.

The number representing the H.T.U. of any preparation having an M.L.D. from 0.010 to 0.099 may be found in this way, while those

TABLE III

	A	B	C	D	E	F	G	H	I
Tr. Strophanthus, U.S.P. 1890.	.00009	.0001	.00011	.00012	.00013	.00014	**.00015**	.00016	.00017
Tr. Strophanthus, U.S.P. 1900.	.000045	.00005	.000055	.000060	.000065	.00007	**.000075**	.000080	.000085
F. E. Digitalis (70 per cent. alcohol)	.0009	.0010	.0011	.0012	.0013	.0014	**.0015**	.0016	.0017
S. E. Digitalis	.00030	.00033	.00037	.0004	.00043	.00047	**.0005**	.00053	.00057
Tr. Digitalis	.009	.010	.011	.012	.013	.014	**.015**	.016	.017
Digitalin....	.00003	.000033	.000037	.00004	.000043	.000047	**.00005**	.000053	.000057
F. E. Squill, U.S.P. 1890.	.00072	.0008	.00088	.00096	.00104	.00112	**.0012**	.00128	.00136
F. E. Convalaria, U.S.P.	.00015	.00017	.00018	.00020	.00022	.00023	**.00025**	.00027	.00028
Strophanthin.	.0000–006	.0000–0066	.0000–0073	.0000–008	.0000–0086	.0000–0093	**.0000–01**	.0000–0106	.0000–0113

M.L.D. of samples	A	B	C	D	E	F	G	H	I
0.010	6.000	6.667	7.333	8.	8.667	9.333	10.	10.667	11.333
0.011	5.454	6.061	6.667	7.273	7.879	8.485	9.091	9.697	10.303
0.012	5.000	5.555	6.111	6.667	7.222	7.778	8.333	8.889	9.444
0.013	4.615	5.128	5.641	6.154	6.667	7.179	7.692	8.205	8.718
0.014	4.286	4.762	5.238	5.714	6.190	6.667	7.143	7.619	8.095
0.015	4.000	4.444	4.889	5.333	5.778	6.222	6.667	7.111	7.556
0.016	3.750	4.166	4.583	5.000	5.417	5.833	6.250	6.667	7.083
0.017	3.529	3.921	4.314	4.706	5.008	5.490	5.882	6.275	6.667
0.018	3.333	3.704	4.074	4.444	4.814	5.184	5.555	5.926	6.296
0.019	3.158	3.508	3.860	4.211	4.561	4.912	5.263	5.614	5.965
0.020	3.	3.333	3.667	4.000	4.333	4.667	5.	5.333	5.667
0.021	2.857	3.175	3.492	3.810	4.127	4.444	4.762	5.079	5.397
0.022	2.727	3.030	3.333	3.636	3.939	4.242	4.545	4.848	5.151
0.023	2.609	2.899	3.188	3.478	3.768	4.058	4.348	4.638	4.927
0.024	2.500	2.778	3.056	3.333	3.611	3.889	4.167	4.444	4.722
0.025	2.400	2.667	2.933	3.200	3.467	3.733	4.	4.267	4.533
0.026	2.307	2.564	2.820	3.077	3.333	3.589	3.846	4.102	4.359
0.027	2.222	2.469	2.716	2.963	3.210	3.457	3.703	3.951	4.197
0.028	2.143	2.381	2.619	2.857	3.095	3.333	3.572	3.810	4.047
0.029	2.069	2.300	2.529	2.759	2.988	3.218	3.448	3.678	3.908

TABLE III.—*Continued*

M.L.D. of Samples	A	B	C	D	E	F	G	H	I
0.030	2.000	2.222	2.444	2.667	2.889	3.111	3.333	3.555	3.778
0.031	1.935	2.151	2.366	2.581	2.796	3.011	3.226	3.441	3.656
0.032	1.875	2.083	2.292	2.500	2.709	2.917	3.125	3.333	3.541
0.033	1.818	2.020	2.222	2.424	2.626	2.828	3.030	3.232	3.434
0.034	1.765	1.960	2.157	2.353	2.549	2.745	2.941	3.137	3.333
0.035	1.714	1.905	2.095	2.286	2.476	2.667	2 857	3.048	3.238
0.036	1.667	1.852	2.037	2.222	2.407	2.592	2.778	2.963	3.148
0.037	1.621	1.802	1.982	2.162	2.342	2.523	2.703	2.883	3.063
0.038	1.579	1.754	1.930	2.105	2.281	2.456	2.632	2.807	2.982
0.039	1.538	1.710	1.880	2.051	2.222	2.393	2.564	2.735	2.906
0.040	1.500	1.667	1.833	2.000	2.167	2.333	2.500	2.667	2.833
0.041	1.463	1.626	1.789	1.951	2.114	2.276	2.439	2.602	2.764
0.042	1.429	1.587	1.746	1.905	2.064	2.222	2.381	2.540	2.698
0.043	1.396	1.550	1.705	1.860	2.016	2.170	2.326	2.481	2.636
0.044	1.363	1.515	1.667	1.818	1.969	2.121	2.272	2.424	2.575
0.045	1.333	1.481	1.630	1.778	1.926	2.074	2.222	2.370	2.518
0.046	1.304	1.449	1.594	1.739	1.884	2.029	2.174	2.319	2.463
0.047	1.277	1.418	1.560	1.702	1.844	1.986	2.128	2.270	2.411
0.048	1.250	1.390	1.528	1.667	1.806	1.944	2.083	2.222	2.361
0.049	1.224	1.360	1.497	1.633	1.768	1.905	2.041	2.177	2.313
0.050	1.200	1.333	1.467	1.600	1.733	1.867	2.000	2.133	2.266
0.051	1 176	1.307	1.438	1.569	1.699	1.830	1.961	2.091	2.222
0.052	1.153	1.282	1.410	1.538	1.667	1.795	1.923	2.051	2.179
0.053	1.132	1.258	1.384	1.510	1.635	1.761	1.887	2.013	2.138
0.054	1.111	1.234	1.358	1.481	1.605	1.728	1.851	1.975	2.098
0.055	1.091	1.212	1.333	1.455	1.576	1.697	1.818	1.939	2.060
0.056	1.071	1.191	1.310	1.429	1.548	1.667	1.786	1.905	2.024
0.057	1.053	1.170	1.287	1.404	1.520	1.637	1.754	1.871	1.988
0.058	1.034	1.150	1.264	1.379	1.494	1.609	1.724	1.839	1.954
0.059	1.017	1.130	1.243	1.356	1.469	1.582	1.695	1.808	1.921
0.060	1.	1.111	1.222	1.333	1.444	1.555	1.667	1.778	1.889
0.061	0.984	1.093	1.202	1.311	1.421	1.530	1.639	1.749	1.858
0.062	0.968	1.075	1.183	1.290	1.398	1.505	1.613	1.720	1.828
0.063	0.952	1.058	1.164	1.270	1.376	1.481	1.587	1.693	1.799
0.064	0.938	1.042	1.146	1.250	1.354	1.458	1.562	1.667	1.771
0.065	0.923	1.026	1.128	1.231	1.333	1.436	1.538	1.641	1.744
0.066	0.909	1.010	1.111	1.212	1.313	1.414	1.515	1.616	1.717
0.067	0.896	0.995	1.095	1.194	1.294	1.393	1.492	1.592	1.691
0.068	0.882	0.980	1.078	1.176	1.274	1.372	1.470	1.568	1.667
0.069	0.869	0.966	1.063	1.159	1.256	1.352	1.449	1.546	1.642

TABLE III.—*Continued*

M.L.D. of Samples	A	B	C	D	E	F	G	H	I
0.070	0.857	0.952	1.048	1.143	1.238	1.333	1.428	1.524	1.619
0.071	0.845	0.939	1.033	1.127	1.221	1.315	1.408	1.502	1.596
0.072	0.833	0.926	1.018	1.111	1.203	1.296	1.388	1.481	1.574
0.073	0.822	0.913	1.005	1.096	1.187	1.278	1.370	1.461	1.552
0.074	0.810	0.901	0.991	1.081	1.171	1.261	1.451	1.441	1.531
0.075	0.800	0.889	0.978	1.067	1.156	1.244	1.333	1.422	1.511
0.076	0.789	0.877	0.965	1.053	1.140	1.228	1.316	1.403	1.491
0.077	0.779	0.866	0.952	1.039	1.125	1.212	1.300	1.385	1.472
0.078	0.770	0.855	0.940	1.026	1.111	1.196	1.282	1.368	1.453
0.079	0.759	0.844	0.928	1.013	1.097	1.181	1.266	1.350	1.435
0.080	0.750	0.833	0.917	1.000	1.083	1.167	1.250	1.333	1.417
0.081	0.741	0.823	0.905	0.988	1.070	1.152	1.235	1.317	1.399
0.082	0.732	0.813	0.894	0.976	1.057	1.138	1.220	1.301	1.382
0.083	0.723	0.803	0.884	0.964	1.044	1.124	1.205	1.285	1.365
0.084	0.714	0.793	0.873	0.952	1.032	1.111	1.190	1.270	1.349
0.085	0.706	0.784	0.863	0.941	1.020	1.098	1.176	1.255	1.333
0.086	0.698	0.775	0.853	0.930	1.008	1.085	1.163	1.240	1.318
0.087	0.690	0.766	0.843	0.920	0.996	1.073	1.149	1.226	1.303
0.088	0.682	0.757	0.833	0.909	0.985	1.060	1.136	1.212	1.288
0.089	0.674	0.749	0.824	0.900	0.974	1.049	1.124	1.198	1.273
0.090	0.667	0.741	0.815	0.889	0.963	1.037	1.111	1.185	1.259
0.091	0.659	0.733	0.806	0.879	0.952	1.026	1.099	1.172	1.245
0.092	0.652	0.725	0.797	0.870	0.942	1.015	1.087	1.160	1.232
0.093	0.645	0.717	0.789	0.860	0.931	1.004	1.075	1.147	1.219
0.094	0.638	0.709	0.780	0.851	0.922	0.993	1.064	1.135	1.206
0.095	0.632	0.702	0.772	0.842	0.912	0.982	1.053	1.123	1.193
0.096	0.625	0.694	0.764	0.833	0.903	0.972	1.042	1.111	1.180
0.097	0.619	0.687	0.756	0.825	0.893	0.962	1.031	1.099	1.168
0.098	0.612	0.680	0.748	0.816	0.884	0.952	1.020	1.088	1.156
0.099	0.606	0.673	0.741	0.808	0.875	0.943	1.010	1.077	1.145

of greater toxicity may be obtained by using a multiple of the number given. For example, if a sample of F.E. Digitalis has the M.L.D. 0.0020 while that of the standard F.E. Digitalis is 0.0012, the sample contains 40 H.T.U. since its toxicity is ten times that used in the first illustration.

It is evident, therefore, that with the data obtained from the assay on frogs one may find in the table the heat tonic units accurately determined for any degree of toxicity.

In a laboratory where samples of every preparation of this series may come in for assay at one time it is inconvenient and, as one can

readily see, unnecessary to have an assay of the corresponding standard for each one, since the only object of testing the standard in comparison with the sample is to determine the resistance of the frogs. For this purpose, therefore, in an emergency any one of the preparations might be used as the standard, because a change in the resistance of the frogs would bring about a proportionate change in the M.L.D. of all the standards. Whatever standard is adopted, however, should be a product least subject to changes in its activity from any cause. Pure crystalline Kombé Strophanthin is the one which seems to meet all the requirements. This product was finally selected and reported at a meeting of the Philadelphia Section of the American Pharmaceutical Association in March, 1911 (Houghton, American Druggist, July 24, Sept. 11).

Since the publication of the above paper the U.S.P. Revision Committee has adopted the use of Ouabain as the standard of comparison for the official "One Hour" method. Ouabain is to be preferred, therefore, as the standard for the 12 hour method because the Ouabain as supplied by the Bureau of Chemistry is uniform in activity and the use of the same standard substance for both methods makes the assay results comparable.

The ninth horizontal column of numbers representing M.L.D. of standard preparations of the digitalis series of heart tonics are those for Kombé Strophanthin. These are enclosed between heavy lines. Kombé Strophanthin contains 100,000 H.T.U. per gram, therefore, when this substance is used as the standard the number of H.T.U. in any sample being tested can be calculated by a simpler formula which is obtained by substituting constants in the one previously given and is merely a rearrangement of it. The formula then becomes

$$\frac{100{,}000 \times \text{M.L.D. strophanthin}}{\text{M.L.D. of sample}} \text{ H.T.U. per c.c. or gm.}$$

which can be used at any time in place of the table. The numbers in the table, however, are accurately calculated, and when available are much more convenient than to make the computation in each case."

3. **Focke's Method.**—With this method the heart is exposed *before* the injection of the drug. The strength of the preparation "V"

being determined by means of a formula in which the weight of the frog is divided by the dose multiplied by the time, in minutes, required to complete the reaction.

Apparatus Necessary for Experiment.—Same as under "one-hour" method.

Animals.—Frogs in good physical condition, which have been in captivity not less than three days and weigh between 25 and 30 gm. They are not to be collected before the end of June and should be kept in tanks supplied with water.

Care of Animals.—See p. 244.

Preparation of Experiment.—Six hours before carrying out the actual standardization several frogs of about standard weight (25 to 30 gm.) should be removed from the tank and placed in jars in the laboratory and kept at a temperature not exceeding 17° C.

Preparation of Solutions.—The solution to be injected is prepared by making a 10 per cent. infusion of the drug.

Method of Injecting.—The injections are made, by means of an all-glass syringe or sharp-pointed pipette, into the two leg lymph-sacs.

Actual Standardization.—An unpithed frog is fastened to the board and the heart carefully exposed in the usual manner without causing any loss of blood; 0.25 c.c. to 0.35 c.c. (about one-fortieth of the weight of the frog) of the infusion is then injected into the lymph-sac of each leg. The heart is then watched and the time required to cause systolic stoppage is noted. This is usually from seven to fifteen minutes. If the heart stops in less then seven minutes, the dose has been too large, while if it continues to pulsate after twenty minutes it has been too small.

Basing the dosage upon the result of this primary experiment, other frogs are injected with larger or smaller doses, as the case may be, until four are found in which the heart has stopped between seven and fifteen minutes. The frogs are then pithed and weighed.

The toxic value of the preparation (V) is then determined by means of the formula

$$V = \frac{P}{d \times t}$$

in which (P) represents the weight of the frog, (d) the amount of the drug injected, and (t) the time in minutes required to complete the reaction.

The relative strength of the preparation tested may then be expressed in percentage by comparing the (V) as determined by the experiment with the standard (V) adopted for that particular drug.

Focke states that the most concordant results are obtained if the experiments are carried out during the months of July, August, and September, but that accurate results for standardization purposes may be obtained at any season of the year if the animals are previously standardized and all subsequent assays corrected accordingly.

(b) **Guinea Pigs: Reed and Vanderkleed's Method.**—This method though official for the standardization of the heart depressants only is perhaps the most convenient and generally serviceable method of valuating both the heart tonics and depressants. The guinea-pig is especially well adapted to assay purposes because of its relative slight variation in susceptibility due to age, sex, temperature, seasons, etc., as compared with the large variation found in frogs. The following quotation is taken from the conclusion of a paper based upon the results of an experiment extending over two years on "Variation in Susceptibility of the Guinea-pig."[1]

"With less than a ten per cent. variation above or below the average, with less than ten per cent. of pigs dying or recovering "out of order" we contend that for all practical purposes, the guinea-pig method affords the simplest and most satisfactory means of standardizing the heart tonic group of drugs, at a very reasonable economic cost, without the necessity of standardizing the test animals, and without need for considering seasonal variations."

Apparatus Necessary for Experiments; Animals; Care of Animals; Preparation of Experiment; Preparation of Solution; Method of Injecting; Method; Actual Standardization.—Same as given under standardization of Cardiac Depressants.

[1] Variation in Susceptibility of the Guinea-pig (Continuation of a previously reported study),[2] by Chas. E. Vanderkleed, Phar. D. and Paul S. Pittenger, Phar. D. Jour. p. 558.

[2] Variation in the Susceptibility of the Guinea-pig to the Heart Tonic Group (Second Paper), by Chas. E. Vanderkleed, Phar. D. and Paul S. Pittenger, Phar. D., Journal of the American Pharmaceutical Association, 11, May, 1913, p. 558.

Standards.—In order to express the percentage results it is necessary to adopt for each drug or preparation assayed a standard minimum lethal dose with which the minimum lethal dose of the preparation being tested may be compared. After long experience we have adopted the following provisional unofficial standards:

TABLE IV

The doses given are per gram body-weight of guinea-pig.

Tinctures	
Digitalis	0.0025
Squills	0.0025
Strophanthus	0.0001
Fluid Extracts	
Apocynum	0.00024
Cactus Grandiflorus	0.01
Convallaria	0.0003
Digitalis	0.00025
Squills	0.00025
Strophanthus	0.00001
Solid Extracts	
Digitalis	0.0000625
Active Principles	
Digitalin	0.000016
Ouabain	0.0000002
Strophanthin	0.000001

The above standards are *not official.* When standardizing official preparations for the trade the official standards and methods must be employed. This method, however, is very useful for checking the results obtained by the official method and for the standardization of the heart tonics which are not official.

(c) **Cats. Hatcher and Brody's Method.** This method consists in determining the minimum fatal dose per kilogram of cat, when the drug is injected slowly into the femoral vein, the standard chosen for the digitalis group being the cat-unit.

Cat-unit.—The amount of crystalline ouabain which is fatal within about 90 minutes, to a kilogram of cat, when the drug is injected slowly and almost continuously into the femoral vein. A cat-unit

is equal to almost precisely 0.1 mg. of crystalline ouabain, or one ten-millionth of the weight of the animal.

Apparatus Necessary for the Experiment.—Accurately graduated syringe or burette; cat-board, two graduated burettes. Operating instruments: scalpels, tweezers, grooved director, hemostat, bulldog clamps, glass seeker, two small glass cannulas, and silk ligatures.

Animals. Cats of medium size (1.5 to 4 kg.) in good physical condition.

Care of Animals. See p. 238.

Preparation of Experiment.—After weighing, the animal is completely anesthetized. This is best accomplished by one of the two following methods:—

1. *Ether Anesthesia.*—The animal is placed in an air-tight box or bell-jar, into which is dropped a sponge saturated with ether; the animal is allowed to remain until all voluntary movements cease. It is then removed from the box or jar and fastened upon the cat-board, the anesthetic now being given on cotton covered with a towel, care being taken to cover the whole mouth of the animal.

2. *Morphine Acetone-chloroform Anesthesia* (Edmunds and Cushny).—The animal is placed in a box 35 cm. long, 18 cm. wide, and 18 cm. deep. The box is furnished with a sliding lid. A V-shaped cut is made in the end of the lid and in the corresponding end of the box, so that the animal may be securely clamped in this opening, allowing the head to protrude. The lid is fixed with a nail and then 40 to 60 mg. of morphine are injected with a hypodermic syringe into the skin of the neck. This is followed by 0.3 cm. per kilogram of acetone-chloroform dissolved in alcohol administered by the stomach-tube. As soon as voluntary movements cease the cat is removed from the box and tied upon the board.

Next make an incision two inches long over each saphenous vein at its junction with the femoral vein and sever the tissues just enough to free about one inch of each. A short cannula of small bore, with rubber connection, is then tied into each saphenous vein close to its junction with the femoral vein and the vein clamped off with a small artery clamp.

Preparation of Solutions.—Two solutions are required—*First*, the standard solution is prepared by carefully dissolving 0.1 mg. of

crystalline ouabain per kilogram body-weight of animal, in a sufficient quantity of normal saline solution to render it possible to measure accurately aliquot portions of this dose.

Second, the preparation to be standardized should also be carefully diluted with normal saline solution. Tinctures, fluidextracts, extracts etc. should be prepared for injection as described under the "One Hour" Frog Method p. 30.

FIG. 14.—Arrangement of apparatus for performing drug assays upon anesthetized cats according to the method of Hatcher and Brody. *a*, burette for injecting the standard ouabain solution; *b*, burette for injecting the unknown preparation; *c* and *c'*, rubber connections; *d*, cannula tied into saphenous vein; *e*, anesthetized cat.

Methods of Injecting.—Two glass syringes, or, better, graduated burettes, may be used for making the injections. One marked *A*, for the standard solution, is fastened to the rubber connection of the cannula in one saphenous vein, while the other, marked *B*, for the solution to be standardized, is fastened to the cannula in the other vein. (See Fig. 14.)

Actual Standardization.[1]—"When crystalline ouabain, amorphous strophanthin, or a preparation of strophanthus is to be tested, it is only necessary to inject the solution from a syringe or burette into

[1] Hatcher and Brody Method, Am. Jour. Pharm., Aug., 1910, p. 362.

the femoral vein until the animal begins to show toxic symptoms. The injection is then interrupted, or continued more slowly until the unmistakable signs of approaching death are seen. These signs are so typical that one is rarely mistaken concerning them. They consist in irregularity of the heart, difficult respiration, convulsions, and frequently a peculiar cry, after which recovery is extremely rare. If death does not occur in a few minutes the injection is continued with extreme caution.

"Other members of the digitalis group may be tested in the same manner, but, as a rule, the results will be somewhat too high, and in that case the necessary correction, usually amounting to about 20 per cent., may be made, or the assay may be made more accurately by a modification of the technique.

"Somewhat more uniform results are obtained if about 75 per cent. of the total amount of the digitalis body is injected in the first fifteen minutes and the remainder in the following hour. These results will still be too high, and we have therefore devised a modification of the method of estimating some of the other digitalis bodies which give results that we believe to be nearly as accurate as those obtained with crystalline ouabain itself.

"Just as the analytical chemist may find it desirable to determine the alkalinity of a liquid by adding an excess of acid and titrating back with an alkali, so we have here been able to obtain more accurate results; in some cases when we inject a measured amount of the digitalis body (tincture or infusion of digitalis or digitoxin) in the first period of ten minutes, and after an interval of twenty minutes continue the injection, substituting a solution of crystalline ouabain for that of the digitalis body under examination, until the death of the animal, or until toxic symptoms appear.

"The difference between the amount of crystalline ouabain actually used to complete the assay and the 0.1 mg. per kilogram of animal (the amount which would have been required in the absence of the digitalis body) represents the activity of the digitalis used.

"The following example will illustrate the mode of computing the activity of the digitalis body tested: A tincture representing 70 mg. of digitalis per kilogram of cat was injected into the femoral vein and after twenty minutes the injection of a solution of ouabain was

begun. The animal died with typical symptoms of digitalis poisoning when 0.0142 mg. of crystalline ouabain per kilogram had been injected. The difference between 0.0142 mg. and 0.1 mg. (which would have been required had the ouabain been used alone) is 0.085 mg., or 85.8 per cent., of a cat-unit; hence, 70 mg. of digitalis equals 85.8 per cent. of a cat-unit, and 81.6 mg. of the digitalis equals one cat-unit."

Standards.—This method is *not official* and there are, therefore, no official standards. Experience has shown, however, that the figures given below indicate what may be taken as tentative standards: (Hatcher, 1912).

TABLE V

The figures represent the number of milligrams that contain one cat-unit; *i.e.*, that kill 1 kg. of cat.

Crude Drugs		Principles	
Squill	575	Adonidin	4.35
Euonymus	475	Digitalein	3.5
Hellborus	100	Convallamarin	1.7
Digitalis	100	Hellborein	1.7
Apocynum	70	Digitalin true	1.5
Convallaria	50	Scillitoxin	0.4
Strophanthus Kombe	3.0	Digitoxin	0.3 0.5
Stroph. hispidus	1.5	Strophanthin Amorph	0.13–0.17
		Ouabain	0.1

Commercial Preparations

Digitalin German	3.6
Digitalin Cryst. Nativelle (Levine, 1920)	0.8
Ouabain Arnaud (Levine)	0.06

(d) **Gold Fish Method.**[1]—This method consists in determining the minimum amount of tincture of digitalis which in 500 mils of "tap-water" will prove fatal to gold fish within 3 hours, the fish being immersed in the solution which is kept at a constant temperature of 22° C.

[1] Preliminary Note on a New Pharmacodynamic Assay Method, by Paul S. Pittenger and Chas. E. Vanderkleed, Jour. A. Ph. A., April 1915, 427.

Preliminary Note on a New Pharmacodynamic Assay Method, (Continuation of a Previously Reported Paper) by Paul S. Pittenger, Jour. A. Ph. A., Nov. 1919, 893.

Apparatus Necessary for Experiment.—Constant temperature bath,[1] 800 c.c. beakers, 500 c.c. volumetric flask, 10 c.c. pipette graduated in tenths and a 2 c.c. pipette graduated in hundredths.

Animals.—Common gold fish about 2 1/2 to 3 inches in length, in good healthy condition.

Care of Animals.—See p. 249.

Preparation of Experiment.—Adjust constant temperature bath so that it maintains a temperature of 22° C. Wash and thoroughly dry six 800 c.c. beakers and label 1 to 6, respectively; accurately pipette to the hundredth of a c.c. 7/10 of the standard dose into the 500 c.c. volumetric flask and fill to the mark with "tap-water;" shake thoroughly and empty into beaker No. 1; five other solutions are similarly prepared containing 8/10, 9/10, 10/10, 11/10 and 12/10, respectively, of the standard, and placed in the beakers 2 to 6, respectively; all six beakers are then placed in the constant temperature bath, together with another larger beaker containing 6 gold fish in plain "tap-water."

Actual Standardization.—After one hour the fish are removed from the large beaker and one is placed in each of the six beakers containing the various dilutions of the drug. In removing the fish from the "tap-water" to the drugged solution, care should be exercised that no water be transferred with them. Note the time that the fish are placed in the drugged solutions. Maintain constant temperature of 22° C. After 3 hours note should be made of those living and those which are dead.

The results of this preliminary test, in which the range of dosage is quite wide, enables the investigator to form some idea as to the strength of the preparation. Basing the dosage upon these results, other series of dilutions are made by progressively increasing or decreasing the strength of the dilutions, as the case may be, still further diminishing the variation between doses, until the smallest amount of tincture in 500 c.c. of water is found which will prove fatal within 3 hours.

The probable M.L.D. (minimum lethal dose) of the preparation, unless it deviates considerably from that of the standard, is generally obtained by one or two series of dilutions.

[1] Pittenger, J., A. Ph. A., Nov. 1916, p. 1260.

In order to determine whether or not this is the true M.L.D. this result is checked by carefully preparing a new series of four dilutions; two with the smallest amount of tincture which was found to kill and two with the largest amount of tincture which did not kill. If, however, any of this last series show irregularities, further correction must be made.

After thus determining the M.L.D. of the preparation its relative strength can be calculated by comparing the M.L.D. of the unknown with the standard M.L.D. by simple proportion.

Standard.—A tincture of digitalis to be of standard strength (tentative *not official*) should have an M.L.D. of 2.85 c.c. when assayed by this method.

The summary of the author's second paper upon the use of gold fish as test animals follows:

1. Variations of less than 2 per cent. in the strength of tincture of digitalis can be accurately determined by the method outlined.

2. Variations due to difference in the rate of absorption appear to be practically eliminated by the use of these animals.

3. The weight of the fish may be disregarded when making tests by this method.

4. Variations in temperature markedly influence the resistance of gold fish to digitalis poisoning.

5. The individual variations in susceptibility of gold fish is much less than that in guinea pigs and frogs.

6. The gold fish method is unquestionably the simplest so far proposed and can easily be carried out by those not especially skilled in the pharmacodynamic art.

7. The inexpensiveness of the assay is decidedly in its favor. Gold fish of the proper size can be purchased wholesale for from 45 to 60 cents per dozen.

8. A sufficient number of animals can be procured at all seasons of the year.

9. Alcohol to the extent of that contained in the U.S.P. tincture does not affect the results.

Although this method affords a simple, accurate method of determining the relative strength of two or more different preparations it has not been developed to the point where it is satisfactory

for use as a general routine method for standardization. Before the method is satisfactory for this purpose the seasonal variation in susceptibility of gold fish must be determined and a suitable "standard substance" adopted by which this variation may be measured and the assay results corrected accordingly.

2. BLOOD-PRESSURE METHOD. *Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Method of Injecting.* Same as given under standardization of epinephrin.

Preparation of Solution.— All preparations to be tested should be diluted with normal saline solution to tincture strength, tinctures and fluid extracts first being freed from the greater part of alcohol by careful evaporation on a water bath and subsequent addition of distilled water until the original volume is restored.

Actual Standardization.— The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure several inches in length the drum is stopped and 1 c.c. of the preparation to be tested is injected into the femoral vein. Another similar injection is given, after allowing from one and one-half to two hours to elapse, in order that the effects of the previous injection may partially pass away. As the action of this class of drugs is prolonged and cumulative, more than two injections should never be given to the same animal, because the first injection always modifies the succeeding injections. Three or four dogs should be used and the average percentage rise of blood-pressure taken as the figure of potency.

The immediate rise produced by subtoxic doses is never marked, in many cases being so slight that a variation of 15 or even 20 per cent. in the size of the dose injected produces no measurable difference in the resultant rise. This method is, therefore, useful only for *roughly* determining the quantitative activity of a preparation, but does not give results sufficiently accurate to permit its being used for standardization purposes.

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CHAPTER IV

STANDARDIZATION OF CARDIAC DEPRESSANTS

Of the various methods proposed for the quantitative determination of the activity of these drugs the guinea-pig method is the only one which gives satisfactory results. This method, has, therefore, been adopted as the official method for assaying aconite and its preparations. It may also be employed for standardizing gelsemium, veratrum and their preparations. As described on p. 43 this method is also very satisfactory for standardizing the cardiac stimulants.

Official Guinea-Pig Method.—This method formerly known as the *Reed and Vanderkleed Method* consists in determining the minimum dose per gram body-weight of guinea-pig that after subcutaneous injection will kill within six hours, at least two of every three guinea pigs injected.

Apparatus Necessary for Experiments.—One Hitchens syringe, pipettes graduated in 1/100 c.c., scissors, scales and a set of weights ranging from 1 to 500 gm.

Animals.—Guinea-pigs in good physical condition and weighing from 275 to 325 gm.

Care of Animals.—See p. 251.

Preparation of Experiment.—The guinea-pigs are prepared for the injection by clipping or shaving the hair from about one square inch of the skin over the abdomen, and painting the exposed portion with 5 per cent. tincture of iodine. The pigs are then weighed and records kept.

Preparation of Solutions.—Crude drugs cannot be administered to the animals as such. Therefore, a tincture or fluidextract must be prepared from the drug and the liquid preparation tested.

Powdered or solid extracts, tablets, etc., should be diluted to tincture or fluidextract strength. In all cases care must be taken

that the solvent used is of the same alcoholic strength as that used in the original extraction of the drug. This is necessary in many cases, to effect complete solution of the active constituents.

The formula used in the preparation of the solid substance should be consulted and the diluent prepared in accordance with the alcoholic content of the original menstruum.

In all cases the preparations should be sufficiently diluted or concentrated, as the case may be, to make the dose injected measure not less than 0.5 c.c. nor more than 4 c.c. Doses of less than 0.5 c.c. are not advisable because the smaller the dose the greater the experimental error. On the other hand doses larger than 4 c.c. are not advisable because the larger the amount injected the slower the absorption. With the injection of exceptionally large doses it is possible for the animal to eliminate some of the drug before it is all absorbed.

As some drugs are many times more toxic than others it is not practical to adopt a definite standard volume for all injections. It is best, however, to have the volume of the injections of each individual preparation practically uniform. For this reason the U.S.P. directs that in the case of Aconite the tincture should be diluted with distilled water to make the dose about 1 c.c.

With drugs such as Gelsemium and Veratrum which are less toxic than Aconite a dose of 1 c.c. is not sufficient to kill the animal. Therefore, a standard dose of a larger volume must be adopted. This is more advisable than concentrating the preparation.

The simplest method for bringing the size of the various injections of the same preparation to an equal volume is to graduate the side arm (C) of the Hitchens syringe (Fig. 15 p. 55) into about four equal volumes. After accurately pipetting, the desired dose into the side arm of the syringe dilute, to the desired graduation, with distilled water from a "wash bottle." All doses of the same preparation should be diluted to the same graduation.

It has not been found necessary to remove the alcohol from the various preparations when testing by this method. Although alcohol is thought to slightly increase the resistance of the animals it does not effect the results as the alcoholic content of the same preparation of the same drug is always practically uniform.

Method of Injecting. The injections are given subcutaneously, in the abdominal region. Figure 15 illustrates a very simple and handy method.

The Hitchens syringe is especially adapted for this work because it allows no possibility of loss while inserting the needle and may be washed with water without being withdrawn.

Method.—Pipette the desired dose of the preparation to be injected, into the side arm (C) of the syringe; while holding the syringe with the side arm down, insert the needle as shown below;

FIG. 15.—Method of injecting guinea-pigs. A, detachable bulb; *B* and *C*, glass body of Hitchens syringe; *D*, needle.

invert the syringe to allow the liquid to run from *A* to *C*; insert rubber bulb (*A*) into the neck of syringe and inject the liquid by applying pressure to the bulb with the thumb; remove bulb; wash the side arm with about 1 c.c. of water from a "wash bottle;" rotate syringe several times, replace bulb and inject wash-water; massage injected liquid away from the point of injection; withdraw needle.

Actual Standardization.—Inject into a series of four guinea-pigs 9/10, 10/10, 11/10, and 12/10 of the standard dose of the preparation to be standardized for each gram body-weight of guinea-pig. The animals are then placed in cages (see page 232) and allowed to

remain for six hours;[1] when they are examined and a note made of those living and those which are dead.

The results of this preliminary test, in which the range of dosage is quite wide, enables the investigator to form some idea as to the strength of the preparation. Basing the dosage upon these results, other series of guinea-pigs are injected with progressively increasing or decreasing doses, as the case may be, still further diminishing the variation between doses, until the smallest amount is found which will prove fatal within six hours. The probable minimum lethal (toxic) dose of the preparation, unless it deviates considerably from that of the standard, is generally obtained by one or two series of injections. In order to determine whether or not this is the true minimum lethal dose, this result is checked by carefully injecting a new series of four pigs; two with the smallest dose that was found to kill, and two with the largest dose that did not kill. If, however, any of this last series show irregularities, further correction must be made.

Figure 16 shows a convenient method of recording the necessary data pertaining to minimum lethal dose (M.L.D.) experiments. This figure may also be used to demonstrate an assay of tincture of aconite. It will be noted that on the first day (3/12) doses were given ranging from 0.0003 to 0.0005 c.c. per gram body-weight of animal; after six hours the results show that all had recovered except two—that which received a dose of 0.00045 c.c. per gm. and that which received 0.0005 c.c. per gm. These results showed that the M.L.D. was between 0.0004 and 0.00045. Therefore on the succeeding day (3/13) doses were given between these two, namely, 0.0004, 0.000425 and 0.00045 c.c. After six hours the results show that the pigs which had received the doses of 0.0004 and 0.000425 c.c. respectively recovered, while the one which received 0.00045 c.c., died, thus showing the M.L.D. to be 0.00045 c.c. per gram body-weight. In order to check these results two more pigs were injected, one with 0.000425 (the largest dose from which a pig recovered)

[1] According to the original Reed and Vanderkleed guinea-pig method the results should be recorded *two* hours after the injection of the drug. Later investigations by the author on a series of 1200 guinea-pigs shows that more concordant results can be obtained by increasing the time limit. The U.S.P. has since officially set the time limit at six hours.

RESULTS OF EXPERIMENTS ON ANIMALS

Number	Substance	Dose per gram	Dilution	Dilution per gram	Description of animal	Weight	Actual dose of dilution	Time	Result and remarks	Date
17683	Tr. Aconite	0.0003	1–5	0.0015	Yellow	320	0.48	8.20	Recovered	3/12
		0.00035	1–5	0.00175	Slate	280	0.48	8.20	Recovered	3/12
		0.0004	1–5	0.002	Black	285	0.57	8.20	Recovered	3/12
		*0.00045	1–5	0.00225	White	325	0.73	8.20	Died	3/12
		0.0005	1–5	0.0025	Cream	310	0.77	8.20	Died	3/12
		0.0004	1–5	0.002	Blk. and white	280	0.56	9.00	Recovered	3/13
		0.000425	1–5	0.00212	Grey	295	0.62	9.00	Recovered	3/13
		*0.00045	1–5	0.00225	Yellow and white	290	0.65	9.00	Died	3/13
		0.000425	1–5	0.00212	Slate and yellow	325	0.69	9.30	Recovered	3/14
		*0.00045	1–5	0.00225	Brown	285	0.64	9.30	Died	3/14

M.L.D. = 0.00045 = 88.8 per cent. of standard.

FIG. 16.—Sample page from the laboratory book showing the results of an experiment on guinea-pigs to determine the minimum lethal dose of a tincture of aconite.

and the other with 0.00045 c.c. (the smallest which had proven fatal). After six hours it was found that the pig which had received 0.000425 c.c. had recovered while the one which received 0.00045 c.c. died, thus checking the former results. The M.L.D. for this preparation therefore was 0.00045 c.c. per gram body-weight. After thus determining the M.L.D. the relative strength of the preparation can be calculated by simple proportion as follows:

The M.L.D. of the unknown preparation = 0.00045 c.c.
The standard M.L.D. for Tr. Aconite = 0.0004 c.c.
(See standards below.)
The percentage strength of the unknown would therefore be
0.00045 : 0.0004 :: 100 : x or 88.8 per cent.

Official Standards.—The following are the official standards for the drugs and their preparations directed to be assayed by this method:

Aconitine U.S.P., administered subcutaneously to guinea pigs, has a minimum lethal dose of not less than 0.00,000,005,5 gm. and not more than 0.00,000,006,5 gm. for each gram of body weight of guinea pig.

Aconite, U.S.P., in the form of the tincture administered subcutaneously to guinea pigs, has a minimum lethal dose, not exceeding 0.0004 c.c. of tincture for each gram of body weight of guinea pig.

Tincture of Aconite, U.S.P., administered subcutaneously to guinea pigs, has a minimum lethal dose of not less than 0.00,035 c.c. and not more than 0.00,045 c.c. for each gram of body weight of guinea-pig.

Fluidextract Aconite, N.F., when assayed biologically, the minimum lethal dose should not be greater than 0.00004 c.c. for each gm. of body weight, of guinea-pig.

Unofficial Standards.—As the guinea-pig method is not official for gelsemium and veratrum it is advisable to adopt some unofficial standard in order to express assay results in terms of percentage. Therefore it is necessary to adopt for each drug or preparation assayed a standard minimum lethal dose with which the minimum lethal dose of the preparation being tested may be compared. After

long experience we have adopted the following provisional standards for our laboratory:

TABLE VI

The doses given are per gram body-weight of guinea-pig.

Tinctures	
Gelsemium[1]	0.01
Veratrum[2]	0.002
Fluid extracts	
Gelsemium	0.001
Veratrum	0.0002
Solid and Powdered Extracts	
Gelsemium	0.00025
Veratrum	0.00005

[1] "The Standardization of Gelsemium," by Paul S. Pittenger, Jour. A. Ph. A., Dec. 1923, 1063.

[2] "The Standardization of Veratrum" by Paul S. Pittenger, Proceedings Penna. Pharmaceutical Ass'n., 1923.

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CHAPTER V

EPINEPHRINE AND PRODUCTS OF THE SUPRARENAL GLAND

Epinephrine is 1, 2-dihydroxy-4[2] methyl-amino ethyl-4[1]-01 benzene, $C_6H_3(OH)_2(CHOH.CH_2NHCH_3)$ a substance with feeble basic properties, obtained from the suprarenal gland of the sheep or other animal. It is normally secreted by the suprarenal gland into the blood-vessels.

Epinephrine acts peripherally on a variety of structures probably by stimulating the sympathetic nerve endings. Its most important therapeutic action consists in a constriction of the blood-vessels, with consequent high rise in blood-pressure, a slowing of the heart due to stimulation of the vagus center, and a direct stimulant and tonic effect on the heart muscle. The effects of a single dose are very fleeting but can be renewed by a fresh injection. Moderate doses given to animals either by mouth or hypodermically have practically no action; the characteristic effects of the drug are, therefore, best elicited by its injection into the vein, when it stimulates the terminations of the sympathetic nerves arising from the lumbar and dorsal regions of the spinal cord.

The contraction of the vessels due to epinephrine can be shown by applying it to a mucous membrane, when the part becomes pale and anemic from the constriction of the vessels; this is well seen when the drug is applied to the congested conjunctiva. That the contraction of the vessels is the principal cause of the rise of blood-pressure may be easily shown by the fact that the volume of the organs, the venous pressure, and the outflow of blood from the veins are all diminished during the rise of the arterial pressure.

The effect upon the circulation may be demonstrated by the following experiment.

Apparatus Necessary for Experiment; Animals; Preparation of Experiment; Preparation of Solutions; and Method of Injection.—

Same as required for the standardization of epinephrine (see page 64).

Experiment.—After all preliminary arrangements have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood-pressure tracing is then started on a slowly revolving drum. After obtaining a tracing of normal pressure about 3 in. in length, inject 0.2 to 0.3 c.c. of a 1 to 10,000 solution of epinephrine into the femoral vein; take continuous tracing until the blood-pressure returns to normal. It will be noted from Fig. 17 that immediately after the intravenous injection of epinephrine the blood-pressure rises sharply due for the most part to constriction of the vessels of the abdominal cavity; as the pressure

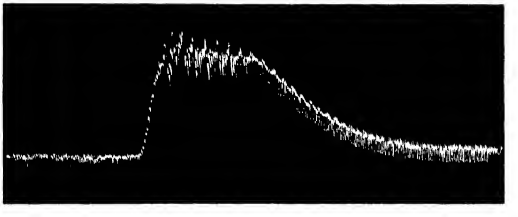

FIG. 17.—Tracing showing effect on blood-pressure of extract of suprarenal gland, which was injected into the femoral vein.

approaches its maximum the heart beats are greatly slowed and strengthened. The slowing is obviously due to excitation of the vagus center produced by the increasing blood-pressure. The increase in strength of the contractions is due to stimulation of the terminations of the accelerator nerves in the heart muscle. After the pressure reaches its maximum it is not sustained but quickly returns to normal accompanied by an acceleration, due to the fall in pressure or to the vagus center becoming exhausted, thus allowing the accelerator stimulation again to gain the upper hand.

Stimulation of the Cardiac Muscle.—The effect of suprarenal extracts upon the cardiac muscle may be demonstrated by perfusing the excised mammalian heart with blood containing epinephrine.

Experiment.—For *Apparatus Necessary; Animals; Preparation of Experiment; Preparation of Solutions;* and *Technique employed* see

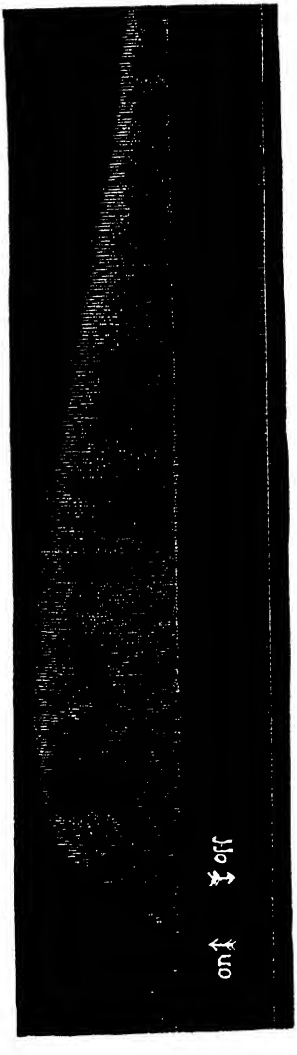

FIG. 18.—Action of 0.0001 per cent. epinephrine hydrochloride on the isolated cat's heart. The heart was giving weak contractions just before receiving the drug. Perfused between the arrows with epinephrine. Time in seconds. (From Greene's Experimental Pharmacology.)

description under "Isolated Mammalian Heart," page 317. First perfuse a cat's heart in the usual way with Loche-blood solution until a normal tracing several inches in length has been obtained, then change to 0.0001 per cent. solution of epinephrine hydrochloride in Loche-blood solution. Take continuous tracing. It will be noted that immediately after application of the drug the tone is markedly increased; if the heart is beating feebly it often happens that the contractions will increase in amplitude by 200 per cent. or more.

Of the various physiologic actions of the gland above mentioned, the effect upon the blood-pressure presents the best means of physiologic standardization.

Official Blood-pressure Method for the Standardization of Epinephrine and Products of the Suprarenal Gland

This assay depends upon the characteristic, transitory, quantitative rise in blood-pressure in dogs, produced by the intravenous injection of sub-maximal doses properly diluted.

Apparatus Necessary for Experiment.—A large kymograph with manometer arranged for taking blood-pressure tracings on continuous rolls of smoked paper; accurately graduated all-glass syringe; large and small scalpels; small, sharp-pointed scissors; grooved director; hemostat; two glass seekers; several bulldog clamps; small glass cannulas; silk ligatures; a dog board.

Animals.—Various animals may be employed, the dog, cat, or rabbit; but dogs of medium weight (8 to 14 kilo) give the best results.

Care of Animals.—See p. 240.

Preparation for Experiment.—First completely anesthetize the animal. Any of the volatile anesthetics, such as ether or chloroform, may be employed, but, since it is of great importance that the blood-pressure does not fluctuate from the action of the anesthetic, it is better to employ one of the following methods for this purpose:

1. Inject subcutaneously 0.01 gm. of morphine sulphate for each kilo of body-weight, supplemented by the use of such a quantity of ether as may be necessary to prevent the pain of the operation. After connecting the artery with the manometer the animal is allowed

to come from under the influence of the ether. No experiments should be begun until at least ten minutes have intervened after the withdrawal of the ether.

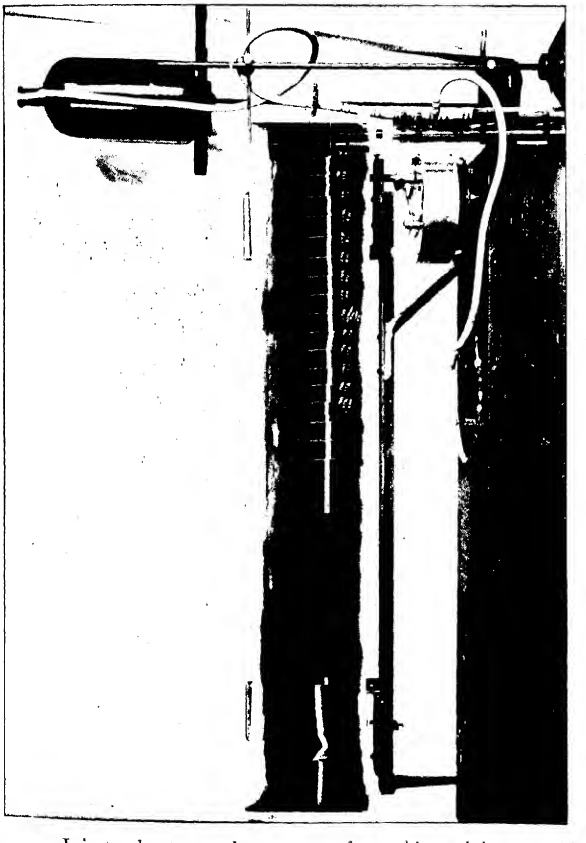

FIG. 19.—Arrangement of apparatus for taking blood-pressure tracing.

2. Inject subcutaneously 0.01 gm. of morphine sulphate per kilo body-weight of animals, and 45 to 60 minutes later give by mouth 1.5 to 2 gm. of acetone chloroform (1.5 gm. for animals weighing 6 to

7 kilos; 2 gm. for those weighing 10 to 12 kilos, and intermediate weights accordingly). The acetone chloroform is prepared for administration by shaking it with 4 c.c. of alcohol until dissolved and then adding 4 c.c. of water and again shaking.

The latter method is especially valuable for this work, as it is easily administered and under its influence the blood-pressure and heart-action remain practically constant for hours. I find, however, in many cases, that the animal does not react in such a way as to give concordant results immediately after the administration of this anesthetic, and therefore advise the following procedure:

3. Administer the anesthetic as set forth above; wait until all voluntary movements have ceased; clip hair from the throat; make

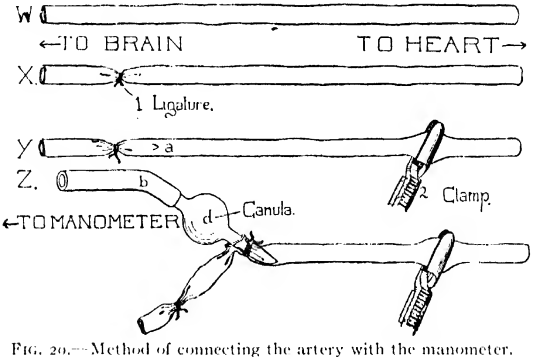

FIG. 20.—Method of connecting the artery with the manometer.

an incision about 2 1/2 in. long; sever the tissues surrounding the carotid artery in such a manner as to free about 3 in. of it, taking care not to injure the vagus, which is found in the same sheath (see page 306). Next make an incision about 2 in. long over the saphenous vein at its junction with the femoral vein and sever the tissues just enough to free about 1 in. of each (see page 332); tie a short cannula of small bore in the saphenous, close to its junction with the femoral; cover both incisions with a piece of gauze saturated with normal saline solution. The trachea may also be exposed and a cannula inserted so that the animal may receive artificial respiration during the course of the experiment if necessary. *The animal*

is then left in this condition for one and one-half to two hours so that the effects of the anesthetic may partially pass off. Before the test is

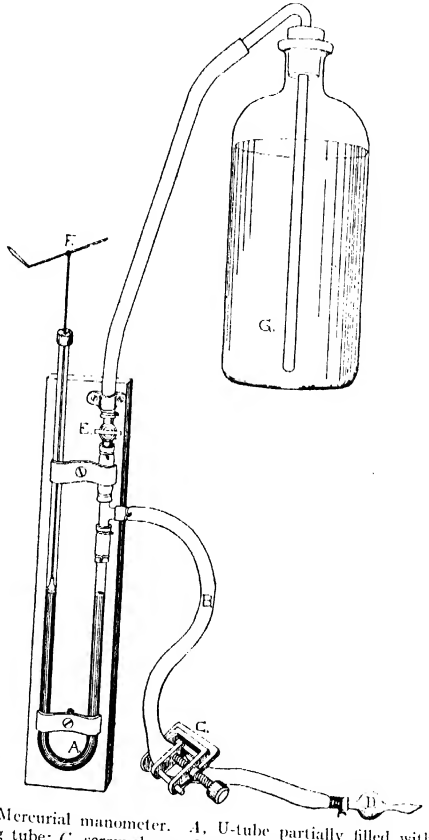

FIG. 21.—Mercurial manometer. *A*, U-tube partially filled with mercury; *B*, connecting tube; *C*, screw-clamp; *D*, cannula; *E*, stopcock; *F*, floater with writing point; *G*, pressure-bottle containing half-saturated magnesium sulphate, or sodium carbonate, solution.

made, in case any muscular movement such as twitching is present, the dog should receive by intravenous injection a sufficient dose of

cuare, but if the animal is deeply anesthetized, this is not necessary. The dog should also receive a sufficient dose of atropine sulphate (from 0.001 gm. to 0.002 gm.) to paralize the vagi, this paralysis being proven by electrical stimulation. After one and one-half to two hours remove the gauze from the neck; lift the exposed part of the carotid artery by means of a glass seeker; tie off that part of the artery leading to the brain (Fig. 20, 1) and close that part leading to the heart with a "bulldog" clamp (2), leaving at least 2 in. between the ligature and the clamp; snip a small V-shaped hole in the artery (*a*), about 1/4 in. from the ligature, with sharp-pointed scissors; make sure that the connecting tube (*b*) and cannula (*d*) are free from air; insert cannula in hole (*a*) and tie the artery fast by means of another ligature (Fig. 20, *Z*). Open *C* (Fig. 21) and *E* in order to fill the portion of the artery between the cannula and the bulldog clamp with the magnesium sulphate solution. This keeps the blood from entering the cannula and thus prevents clotting; close *E*; and close *C*; remove clamp from artery; slowly open *C* until the floater *F* makes an excursion of about 2/8 to 3/8 in. It should then be made to write on a drum which will revolve slowly about 1 1/2 to 2 in. per minute.

Preparation of Solutions.—Prepare a *standard solution of epinephrine hydrochloride from the standard epinephrine* (as supplied by the U. S. Dept. of Agriculture, Bureau of Chemistry) by dissolving 0.050 gm. of epinephrine in 5 c.c. of tenth-normal hydrochloric acid, and dilute this to 50 c.c. by the addition of distilled water, thus making a 1 in 1000 solution. For the assay, add 1 c.c. of this 1 in 1000 solution to 99 c.c. of physiological solution of sodium chloride. (See p. 71.) This dilute solution (1 in 100,000) must be freshly prepared when needed. On account of the possibility of deterioration, the 1 in 1000 solution must have been recently prepared. It will keep for a short time if preserved in amber-colored bottles in a refrigerator, but it must be discarded if any signs of deterioration, such as discoloration, are observed.

The *solution of the epinephrine to be tested* should be prepared in the same manner as the standard solution as described above.

Solution of Desiccated Suprarenals.—If a sample of desiccated suprarenals is to be tested a solution should be prepared by adding

1 gm. of the finely powdered sample to 100 c.c. of distilled water containing 10 c.c. of diluted hydrochloric acid. Allow this mixture to macerate during twenty-four hours, shaking it frequently during that time, and then filter through a dry filter. It is then ready for use.

The preparation to be standardized should be carefully diluted with physiological solution of sodium chloride to the same strength, as nearly as may be estimated, of that of the standard used, or of such strength as may be readily diluted in case the primary injection is found to produce too marked a rise in the blood-pressure.

Method of Injecting.—The injections may be made either in the jugular or the femoral vein. The latter is preferable because it is located farther from the heart, thus giving the preparation injected

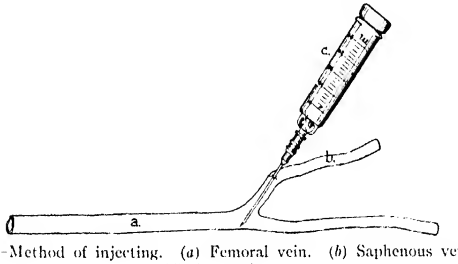

FIG. 22.—Method of injecting. (*a*) Femoral vein. (*b*) Saphenous vein. (*c*) All-glass syringe.

an opportunity to diffuse more thoroughly with the blood before reaching the heart.

The saphenous vein is lifted and held with a pair of tweezers while the needle of the all-glass syringe is inserted far enough through the cannula in the saphenous vein to allow the point to project into the femoral vein (Fig. 22). After injecting the preparation withdraw the needle and quickly clamp saphenous vein with a bulldog clamp.

The advantage of this method is that, although clamping off the saphenous vein after withdrawing the needle causes clotting, the preparation injected is carried to the heart by means of the main current of blood in the femoral vein.

Another convenient method is to tie a cannula of small bore, with rubber connection, into each saphenous vein close to its junction

with the femoral vein. Two graduated burettes may then be used for making the injections. One marked *A*, for the standard solution, is fastened to the rubber connection of the cannula in one saphenous vein, while the other marked *B*, for the solution to be standardized is fastened to the cannula in the other vein. (See Fig. 14, page 46.)

Actual Standardization. (*a*) *Determination of the Proper Dose of the Standard Solution.*—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure, about 3 in. in length, the drum is stopped. Inject the standard solution in a dose of 0.1 c.c. per kilo. The rise in the blood-pressure

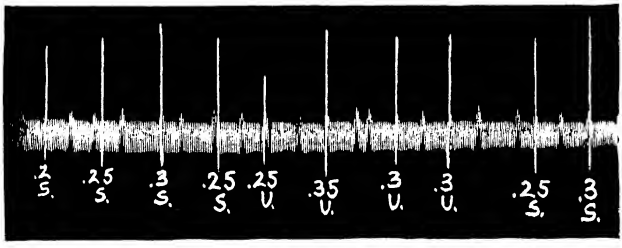

FIG. 23.—Chart shows the method of recording results during an assay of epinephrine. The first three injections demonstrate the sensitiveness of the method by showing variations in the resultant rises produced by differences in the dosage of 0.05 c.c. of a 1–10,000 solution or the equivalent of 0.000005 gm.

should be about 40 to 60 mm. of mercury (equivalent to 20 to 30 mm. rise of the writing-point of the floater in a U-shaped manometer), and it should be submaximal. To determine the latter, a second injection of 0.175 c.c. per kilo should be made after allowing the drum to revolve about 1 in., which should show a higher rise. If the dose of 0.15 c.c. per kilo does not give a submaximal rise of at least 40 mm., or if it gives a maximal rise, the dose should be increased or reduced, respectively, until the standard effect is obtained, and this dose should be considered the "standard dose." The injections should be made at about the same rate, and an interval of at least three minutes should elapse after the blood-pressure has returned to normal before another injection is made.

The "standard dose" should be large enough to cause *almost* the maximum rise, because the larger the amounts of active principle compared the more accurate the results. It is well known that the stronger the stimulus the more nearly in accord are the results obtained.

(*b*) *Comparison of the Unknown with the Standard Solution.* A "standard dose" of the unknown solution is injected and the rise of pressure compared with that produced by the "standard dose" of the standard solution. If the difference is very great the unknown solution is strengthened or diluted. The size of the injections is then increased or decreased until that dose of the unknown solution is found which will cause the same rise of pressure as that caused by the "standard dose" of the standard solution. Occasional injections of the standard solution should be made to insure constancy in the reaction of the animal. Final equality is tested by injecting alternately the standard and unknown solutions until the average rise of several consecutive injections is practically equal.

Complete and Abbreviated Tracings.—Two kinds of tracings may be obtained—complete ones (Fig. 17)—when the drum is kept in constant motion, and abbreviated ones (Fig. 23), when the drum remains at rest until the reaction is complete. Abbreviated tracings which give only the maximum blood-pressure obtained from each injection are usually sufficient.

In measuring the tracings it must be remembered that the real rise in blood-pressure is *twice* that which is recorded, since there are two sides to the U-tube and the needle only moves through a space that represents one-half of the difference of level between the mercury in the two sides.

It is understood that no calculations are to be made from relative size of rises caused by similar doses, but always from similar rises, the relation being determined by the size of the dose. More accurate results are obtained if the systolic pressure is alone considered than if the average pressure half-way between systole and diastole is taken for the measurement.

Official Standard.—*Solution Epinephrine Hydrochloride,* diluted with physiological solution of sodium chloride in the proportion of one part of the solution of Epinephrine Hydrochloride to 99 parts

of the salt solution, and injected into dogs by the method described below, produces a rise in the systolic blood pressure of the dog corresponding to that produced by an equal amount of the standard solution of Epinephrine Hydrochloride prepared as directed below.

Unofficial Standard.—*Suprarenalum Siccum*, assayed biologically 1 gm. of Dried Suprarenals contains the equivalent of ten milligrammes of laevo-methylaminoethanol-catechol.

Accuracy of Method.—This method is the most sensitive of all the biologic assay methods. Variations of only 0.05 c.c. of a 1 to 10,000 solution of epinephrine or the equivalent of 0.00,000,5 gram of epinephrine can be accurately measured.

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CHAPTER VI

ERGOT

Ergot is the sclerotium of Claviceps purpurea replacing the grain of the rye (secale cereale). It is of great importance in therapeutics and also in toxicology, as wide-spread epidemics of disease have resulted from eating bread made of rye which has been infected with the fungus.

The chemistry of ergot has been the subject of a large number of investigations, which have been attended with little success until Barger, Dale and their co-workers isolated the three following substances:

1. **Ergotinine,** $C_{35}H_{39}O_5N_5$, is almost inert, but its hydrate, ergotoxine, $C_{35}H_{41}O_6N_5$, has a powerful action on the tissues.

2. **Tyramine or hydroxyphenylethylamine,** $OH.C_6H_4.CH_2CH_2NH_2$ has an important stimulating effect on the heart causing an increase in both the strength and the rate, resulting in a rise in blood-pressure.

3. **Isoamylamine,** $(CH_3)_2CHCH_2CH_2NH_2$, is present in amounts too small to influence the general action of the drug.

In practical medicine ergot is generally administered either in the form of the fluid, solid or powdered extract, and we will, therefore, treat only of the *action of the drug as a whole.*

Ergot is very readily absorbed and on reaching the blood exerts its specific effects on non-striated muscle, directly or indirectly, throughout the body. Its various actions may be summarized as follows:

Summary of Actions of Ergot[1]

"1. *Stimulation of unstriped muscle,* partly central, but mainly peripheral, the action being exerted in the ganglionic cells or preganglionic endings. This in turn produces:

[1] Sollmann: Text-book of Pharmacology.

"2. *Contractions of the uterus*, especially when pregnant (leading to abortion); these are intermittent with small doses, tonic and persistent with large doses.

"3. *Vasoconstriction*, differing in extent in different areas, especially powerful in the pulmonary vessels.

"4. With large doses, and in susceptible animals, this leads to *gangrene*, especially in peripherally situated organs.

"5. When rapidly injected, a primary depression and secondary stimulation of the *cardiac muscle*.

"6. *Vomiting* and *increased peristalsis*.

"7. The changes of the circulation leads to affection of the *central nervous system*. These are necessarily variable.

"8. Large doses *paralyze the vasoconstrictor endings*."

The following experiments demonstrate the principal actions of the drug.

Experiment. (a) **Effect of Ergot on the Blood-pressure.**—Prepare the animal according to directions given under Epinephrine Standardization (see page 64); take tracing of normal pressure about 3 in. in length; inject about 0.08 c.c. per kilo of fluid extract of ergot and take continuous tracing until pressure returns to normal.

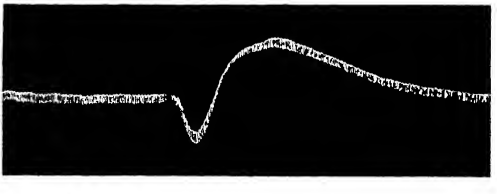

FIG. 24.—Effect of ergot on the blood-pressure.

An intravenous injection of an active preparation of ergot is immediately followed by an abrupt rise in blood-pressure, either with or without a primary fall. The primary fall in pressure is probably due to such impurities as choline; therefore the better the drug the less the fall. The primary fall of pressure is not seen if the drug is injected subcutaneously. The rise in pressure is to be ascribed to stimulation of the constrictor nerve terminations in the vessel walls and is strictly analogous to that observed under epinephrine. (See

page 62.) The sharp rise in pressure is followed in a few minutes by a slight fall, the pressure still remaining, however, if the dose has not been too large, well above normal. If the dose has been too large it produces toxic effects which cause the rise to give way to a fall which carries the pressure below the normal. If the dose be very large and the fall of pressure is not recovered from, progressive paralysis of the vasomotor apparatus and heart occurs.

Experiment. (b) **Effect of Ergot on the Heart.** For apparatus necessary, animals, preparation of experiment, preparation of solutions, and technique employed see description under "Isolated

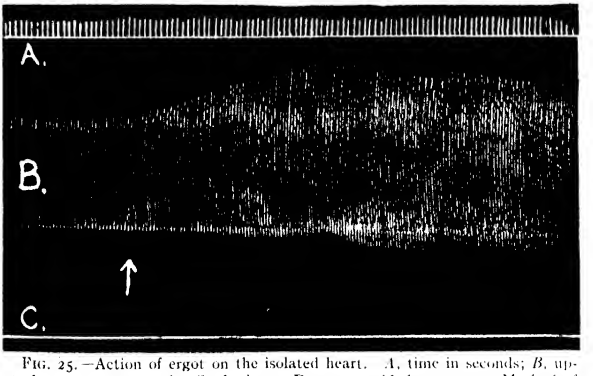

FIG. 25.—Action of ergot on the isolated heart. *A*, time in seconds; *B*, upstroke represents systole; *C*, abscissa. Drug was added at arrow. Method of Langendorff.

Mammalian Heart," page 317. First perfuse heart in the usual manner with Loche-blood solution until a normal tracing several inches in length has been obtained; then change to the drug solution. Allow it to act for about five minutes. Take a continuous record. It will be noted that the heart is decidedly and directly influenced by the ergot—it beats more vigorously, its systole is more complete, and its output is considerably increased. This cardiac effect must contribute to the rise of blood-pressure. It is not yet determined whether this change in the heart is due to direct action on the muscle or to a stimulation of the accelerator myoneural junction. Slowing of the heart is frequently seen after an injection of ergot, and this it

is claimed arises from stimulation of the vagus center by the high blood-pressure and not from the direct action of the drug. This slowing is sometimes so marked that it partially conceals the effect of the vaso-constriction on the blood-pressure tracing.

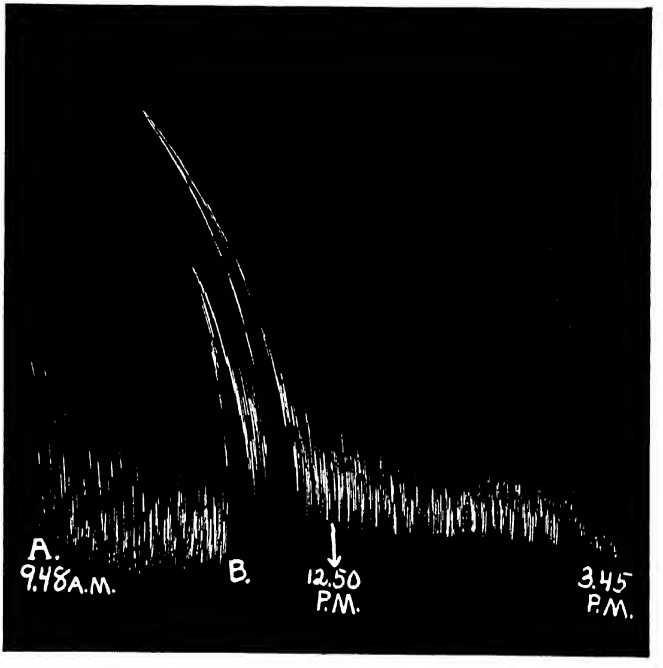

FIG. 26.—Effect of ergot upon the movements of an isolated uterus. Contraction moves the lever upward. *A* to *B* = normal contractions. At *B*, 0.5 c.c., of fluid extract ergot was added.

Experiment. (c) **Effect of Ergot on the Uterus.** For apparatus necessary, animals, preparation of experiments, preparation of solutions, method of injecting, and technique employed, see "Isolated Uterus Method," page 91. After all preliminary arrangements are completed and the writing lever is brought in contact with a very

slowly revolving smoked drum a normal tracing should be taken. A dose of about 0.5 c.c. of F.E. ergot should then be added to the Locke-Ringer's solution in which the uterus is suspended and a continuous tracing taken. If the preparation given contains only a

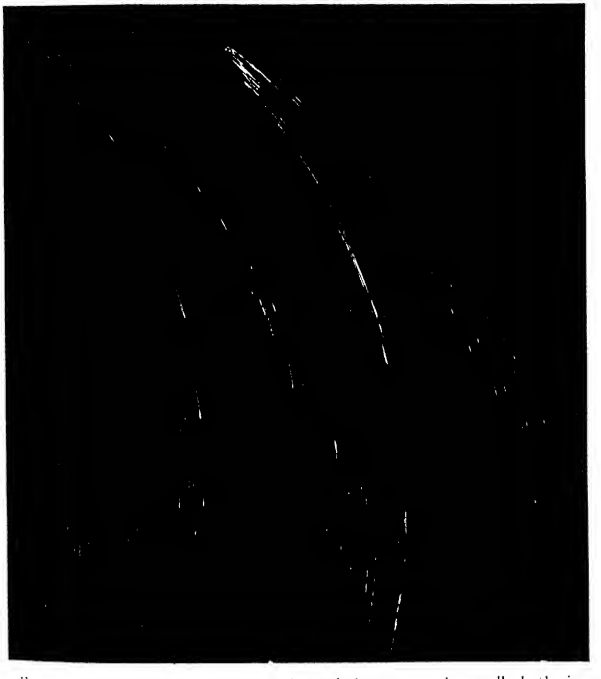

FIG. 27.—Demonstrates the two motions of the uterus—the small rhythmic contractions and the tonic contractions.

small amount of activity it will merely accelerate and strengthen the small rhythmic contractions; on the other hand, should it be a potent extract it will produce a powerful contraction followed by a slow relaxation. (See Fig. 26.) This action is the most important effect of ergot as this drug is mostly employed on account of its

effects on the uterus, which effects are primarily produced by its stimulation of the motor myoneural junction of the hypogastric nerves, while the inhibitory nerves are less strongly affected.

There are two motions to the uterus:[1] the small rhythmic contractions and the tonic contractions. Ergot in full medicinal doses exerts its influence not by materially increasing the normal pains of labor, but by causing a tetanic, tonic, unyielding uterine spasm which drives all before it. In very small doses it may assist the normal contractions without causing them to become tetanic. It is said that after the administration of small therapeutic doses the tonus alone is increased and the movements of the uterus are not excited; but it is certain that *active* ergot in moderately large doses increases both the peristaltic movements and the tonus.

Figure 27 demonstrates the two motions of the uterus. It will be noted that the small rhythmic contractions are superimposed upon the curves produced by the tonic contractions. In order to show this point more clearly the drum was allowed to revolve at the rate of one revolution per hour, or ten times as fast as in Fig. 26.

Standardization of Ergot

There are three principal methods available for the biologic standardization of ergot:—

1. The cock's-comb method.
2. The blood-pressure method.
3. The uterine method—(a) *in situ;* (b) isolated.

1. **The Official Cock's-comb Method.**—This method consists in determining the minimum amount of solution of ergot necessary to cause the same degree of bluing in the cock's-comb and wattles as that produced by a given amount of a standard preparation, when intramuscularly injected.

This method was formerly regarded as very unreliable because of the difference in susceptibility of various cocks. Edmunds was originally unfavorably impressed with this method and discarded

[1] Stewart and Pittenger: "The Application of Some Muscular Tissues Adapted to Physiologic Standardization," Monthly Cyclopedia and Medical Bulletin Sept., 1913.

it, but after more extensive experience he stated in a paper with Hale,[1] that accurate results may be obtained by this method if only white Leghorn cocks are used, since the common barnyard fowl varies too greatly in its reaction.

Although the relative strength of two preparations may be determined quite accurately by this method, it was not well adapted to standardization work previous to its adoption as the official method. This was due to the fact that the method requires the use of a standard preparation with which the strength of the unknown is compared.

This made the standard dependent upon the keeping qualities of a stock galenical. Any deterioration, therefore, in the standard preparation resulted in a lowering of the standard for all subsequent preparations. Also as each worker was compelled to prepare his own standard there was no uniformity of strength between the standards used by the various manufacturers.

Later investigations, however, have shown that practically all of the deterioration takes place during the first three to six months also that fluidextract of ergot may be kept practically permanent in vacuo.[2] This eliminated the first objection to the method. The second objection is eliminated by the fact that the U.S.P. Revision Comittee, before adopting the method, made arrangements whereby the Bureau of Chemistry, U. S. Department of Agriculture prepares and supplies the various standards properly aged and adjusted to all manufacturers throughout the country. This makes it possible for all manufacturers to adjust their preparations to the same standard.

Apparatus Necessary for Experiment.—Accurately graduated syringe for making intramuscular injections and scales with weights up to 2500 gm.

[1] Edmunds and Hale, Bull. No. 76, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv.

[2] Investigations by Pittenger and Vanderkleed,[3] indicate that ergot fluid extract may be kept *in vacuo* without deterioration. This fact makes it possible to preserve standards with which to compare new lots of the drug and their preparations.

[3] A New and Reliable Method for the Preservation of Ergot Preparations, by P. S. Pittenger and C. E. Vanderkleed, Jour. A. Ph. A., August, 1912.

Animals.—Normal, single-comb, white Leghorn cocks, which are less than eighteen months of age, and weigh approximately 2 kilograms. The cocks should be of as nearly the same size and age as possible.

Care of Animals.—See p. 270.

Method of Injecting.—The injections are made deep into the breast muscle by means of an accurately graduated glass syringe.

Preparation of Solutions.—All preparations to be tested should be carefully diluted until 1 c.c. of the dilution represents 1 gram of drug.

Standard Fluidextract of Ergot. The *official* description of the standard as supplied by the Bureau of Chemistry, U. S. Dept. of Agriculture is given on page 83.

Preparation of Experiment.—The fowls should be allowed to fast for twenty four hours previous to making the test. Water should be allowed. Immediately after receiving a lot of cocks for testing purposes their individual susceptibility to the "Standard Fluidextract" should be determined. Each cock should be numbered with leg bands (see Fig. 140) and then injected with varying amounts of the *standard*, at intervals of three to four days, until the smallest amount per kilogram body-weight of animal is determined that will produce a standard darkening of the comb. These amounts are recorded and used for comparison with the amounts of the unknown required to produce the same effects upon the same cocks. The susceptibility of each cock to the "standard" should be checked at least every two months.

Actual Standardization.—Inject a series of three cocks of known susceptibility to the standard, with 0.4 c.c., 0.5 c.c., and 0.6 c.c. respectively, of the unknown preparation, per kilogram body-weight of animal. The cocks are then placed in cages and allowed to remain for one to one and one half hours. The maximum darkening or cyanosis seems to be reached in about that time. The results of this preliminary test, in which the range of dosage is quite wide, enables the investigator to form some idea as to the strength of the preparation. Basing the dosage upon these results, other series of cocks are injected with progressively increasing or decreasing doses, as the case may be, still further diminishing the variation between doses, until the smallest amount is found which will produce approximately

the same intensity of action (darkening of the comb) as was shown by the color of the comb of the same rooster when previously injected with the standard. The probable "Minimum effective dose" of the preparation, unless it deviates considerably from that of the standard, is generally obtained by one or two series of injections. In order to determine whether or not this is the true "Minimum effective dose," this result is checked by carefully injecting a new series of two cocks; one with the smallest dose that produces the standard effect, and one with the largest dose that did not produce darkening of the comb. If, however, the results of this last series do not check the previous results, further correction must be made.

After thus determining the "Minimum effective dose" of the unknown preparation its relative activity is calculated by comparing it with the "Minimum effective dose" of the standard preparation, as previously determined for each individual cock. The same cock may be used for several tests. In fact they may be used until they pass the age limit of 18 months. The same cock, however, must not be used for testing purposes at shorter intervals than two weeks. It should not be considered sufficiently accurate if the first injections of the standard and of the unknown preparations produce the same degree of activity, because it is believed that individual variation in different birds might be sufficiently great to interfere. This variation can be reduced to a minimum, if the order of injection on the succeeding series be reversed until each preparation has been given to each of several birds in turn. The results are then based on the average of the several injections. The final comparisons demand careful selection of the birds to be injected. A choice of two birds of about the same size and which had previously reacted to equal amounts of the standard is advisable. With a little practice it is easy to compare the intensity of the discoloration in the two cocks' combs and to so regulate the dose of the drug given as to produce approximately the same degree of reaction. The reaction should not be sufficiently marked to produce a darkening of the entire comb and wattles. If the reaction is too marked the comb may not entirely return to normal. The reaction should be just sufficient to darken the *tips* of the comb and produce a small dark spot (about 1/2 in. in diameter in the wattles).

Variations may further be avoided by using only such roosters as *react alike to a standard preparation, and also by constantly changing the order of injections so that the same cock shall not receive the same specimen of the drug twice in succession.* An objection to this method, however, is the fact that the personal equation plays an important part in the assay, since the accuracy of the test depends largely upon the experience of the operator and his ability in determining just when the coloration produced by the unknown equals that produced by the standard. In the hands of an experienced operator, however, results may be obtained which will show, with fair accuracy, the relative value of any preparation of ergot.

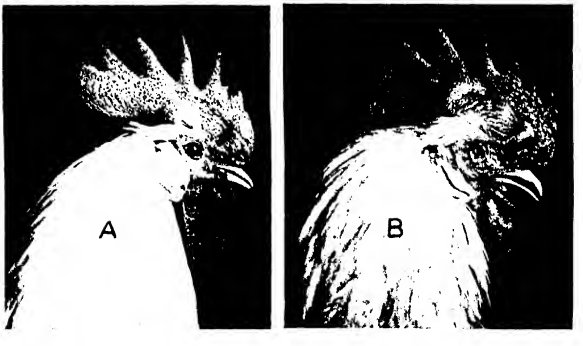

FIG. 28.—Represents the heads of two roosters. *A* is normal and is given for the sake of comparison. *B* shows the darkening of the comb and wattles, after an injection of active ergot.

Official Standards.—The following are the official standards for ergot and its fluidextract which the U.S.P. directs to be assayed by this method:

Ergot, in the form of the fluidextract, administered by intramuscular injection to single-comb, white Leghorn cocks, in doses not exceeding 0.5 c.c. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of a standard fluidextract of ergot, prepared as directed under "Standard Fluidextract of Ergot."

Standard Fluidextract of Ergot.—The U.S.P. directs that the standard fluidextract of ergot supplied by the U. S. Department of Agriculture, Bureau of Chemistry should be prepared as follows: Prepare a composite fluidextract, representing at least ten different lots of ergot, conforming to the official botanical description. This standard fluidextract, which must be aged for at least six months before being standardized by the method described in the preceding paragraph, and must be preserved in a vacuum, when administered by intramuscular injection in doses not exceeding 0.5 c.c. per kilogram body weight of cock, produces darkening of the comb of a single-comb, white Leghorn cock which is less than 18 months of age, and which weighs approximately 2 kilograms.

Fluidextract of Ergot, administered by intramuscular injection into single-comb, white Leghorn cocks, in doses not exceeding 0.5 c.c. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of the standard fluidextract of ergot prepared as directed under *Ergota.*

2. **Blood-pressure Method.**—This method depends upon the characteristic, quantitative rise in blood-pressure in dogs, produced by the intravenous injection of solutions of the drug. The method consists in determining the average rise in pressure produced by three injections, of equal size, administered at one hour intervals, to each of two or three dogs.

This is a very convenient and generally serviceable test. The technique involved is comparatively easy and the effects may be graphically portrayed and accurately measured, thus giving a definite quantitative standard.

Apparatus Necessary for Experiment; Animals; Care of Animals; Preparation of Experiment.—Same as required for the standardization of epinephrine. (See page 64.)

Preparation of Solutions.—All preparations to be tested should be carefully diluted until 1 c.c. of the dilution represents 1 gm. of drug.

Method of Injecting.—Same as that given under Epinephrine Standardization.

Actual Standardization.—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal

pressure about 3 in. in length, the drum is stopped. An injection of 0.04 c.c. (gm. of drug) per kilo of the preparation to be tested is then given and the blood-pressure observed for ten or fifteen minutes. In most cases the injection is followed by a rapid primary fall in pressure, succeeded by an almost equally rapid rise to normal or above—dependent on the strength of the preparation. In order to facilitate the measuring of the myograms, the drum is made to revolve a short distance as follows:

1. When the point is reached where the blood-pressure is about to rise after its primary fall. Fig. 29 (A).

2. Five minutes after the injection. Fig. 29 (5).

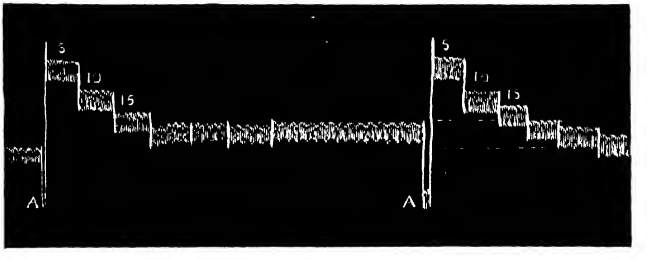

FIG. 29.—Abbreviated Tracing showing method of recording results during an ergot assay. The Figs. 5, 10 and 15 represent the number of minutes after the injection.

3. Ten minutes after the injection. Fig. 29 (10).

4. Fifteen minutes after the injection. Fig. 29 (15).

If the first injection causes a fall in pressure of more than 35 mm., or a rise of more than 48 mm., the dose should be reduced to 0.02 c.c. per kilo. If the fall is less than 24 mm. and the rise less than 24 mm. the dose should be doubled; otherwise, the same dose is repeated.

Three injections are given to one animal, allowing from 60 to 90 minutes to elapse between each injection in order to allow the effects of the previous injection to pass off.

Use of Several Animals.—As dogs vary in their susceptibility to ergot it is necessary to use several animals for check purposes. Three or four injections of the same preparation should be made

into each of three or more dogs and the average rise in pressure taken as the figure of potency. It happens sometimes that one dog out of a series of three or four may prove a decided exception to the others in the way of increased or decreased susceptibility to the drug. The results obtained from such an animal should be discarded.

Standard.—In order to express the activity of the preparation in percentages it is necessary to adopt some provisional standard with which to compare the unknown preparation. After much experience we have adopted the following standard for our laboratory:

"0.08 c.c. of the fluid extract per kilo should cause a rise of blood-pressure of 30 mm."

An objection to this method is that mongrel dogs, such as run at large in the city, differ considerably in their reaction, but, by using two or three dogs for each preparation of ergot, this source of error is considerably reduced.

Another objection to this method has been the lack of proof as to whether or not the action of ergot upon the circulation parallels the action upon the uterus. This is an important factor since the blood-pressure method is rather extensively used for standardization purposes, its employment being supported by statements that the characteristic effect of ergot is a stimulation of all unstriped muscle tissue of the body, and that the changes in the circulation, in the intestines and in the uterus are but a part of one general action. The employment of this method has further been supported by the fact that all the substances which have been suggested by various workers as the active principles of ergot have produced stimulation of the blood-vessels as well as of the uterus. Investigations[1] by Pittenger and Vanderkleed, however, tend to prove that a parallelism does exist between these two actions.

Among the various methods employed for physiologic standardization, blood-pressure tests consume a comparatively great amount of time. This is especially the case with the blood-pressure method for ergot, as it is necessary to check the results on two or three dogs, and,

[1] "A New Uterus-contracting Method of Testing Ergot, with Comparison with the Blood-pressure Method," by Paul S. Pittenger, Phar. D., and Chas. E. Vanderkleed, Phar. D., Jour. A. Ph. A., July, 1914, p. 925.

due to accumulative action, it is also necessary to allow from one to one and one-half hours to elapse between injections. With the usual method of using one manometer and kymograph (see Fig. 19, page 65) only one animal can be used at a time, and it therefore requires the greater part of two days to assay one sample of ergot in

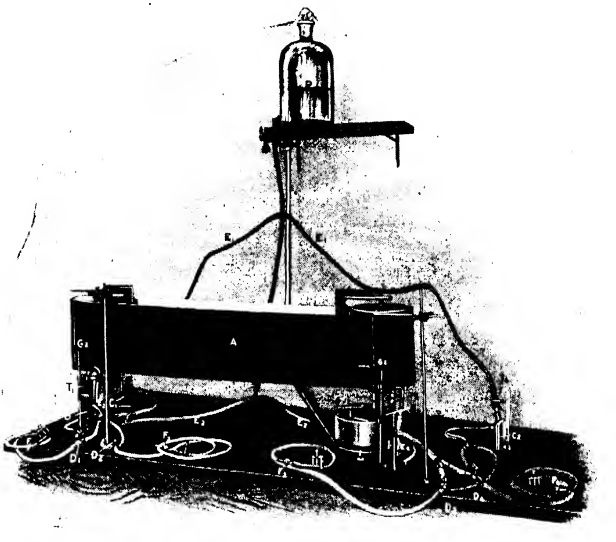

FIG. 30.—Kymograph arranged for making blood-pressure tests on four animals at one time. *A*, long paper kymograph; B_1 and B_2, manometers with writing points; G_1 and G_2, writing points; C_1 and C_2, dummy manometers, without writing points; D_1, D_2, D_3 and D_4, three-way stopcocks; E_1, tubes used for securing pressure in manometers C_1 and C_2; E_2, tubes used for securing pressure in dummy manometers B_1 and B_2; F_1, F_2, F_3 and F_4, cannula; H_1, H_2, H_3 and H_4, connecting tubes; and T_1, T_2, T_3 and T_4, stopcocks.

duplicate, unless several kymographs and manometers are employed. Therefore, in laboratories where considerable numbers of ergot assays are handled and in those where economy of space is essential, it is advisable to employ the following **"Improved Form of Kymograph,"**[1]

[1] "An Improved Form of Kymograph," by Paul S. Pittenger, Phar. D., Journal of the American Pharmaceutical Association, Dec., 1913, p. 1498.

with which it is possible for one man to run blood-pressure tests on four animals at the same time, and record all the tracings on one kymograph without their interfering with each other. This enables one operator to assay at one time with one kymograph two samples of ergot in duplicate, or, he can assay at the same time one sample of ergot in duplicate and one sample of adrenal extract.

Figure 30 shows the arrangement of the apparatus.

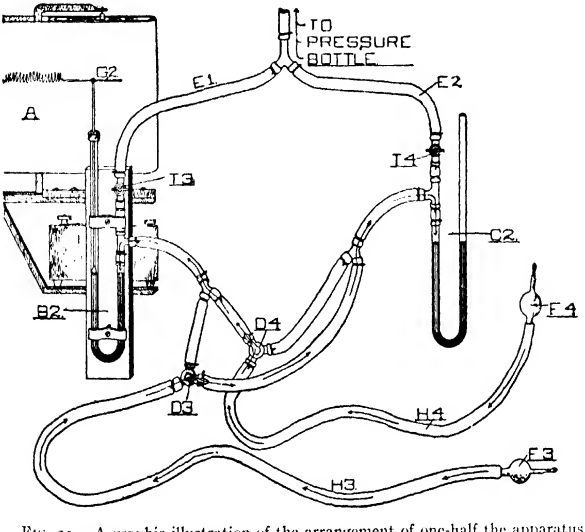

FIG. 31.—A graphic illustration of the arrangement of one-half the apparatus shown in Fig. 30. The letters and figures used in this illustration correspond to those used in Fig. 30.

Description of Method of Employing Apparatus.— The animals are prepared for blood-pressure experiments as given under epinephrine standardization. (See page 64.)

Each of the four cannulæ (F_1, F_2, F_3 and F_4) is then tied into the carotid artery of a dog. Pressure is obtained within the various tubes from the pressure bottle (P) by opening the cocks T_1, T_2, T_3 and T_4 (T_2 invisible). It will be noted from Fig. 29 that each con-

necting tube H_1, H_2, H_3 and H_4, terminates in a three-way stopcock which enables the operator to connect it with either a manometer which writes on the smoked drum, or with a "dummy" manometer.

To assay two samples of ergot it is merely necessary to use two dogs on one end of the kymograph for one sample and two on the other end for the other sample. The three-way stopcocks are arranged in such a manner that one dog on each end records its pulsations upon the revolving drum while the other pulsates against a "dummy" manometer. Inject the proper dose of fluid extract of ergot into the dog which is recording its blood-pressure on the right-hand side of the kymograph; allow the drum to revolve five, ten and fifteen minutes after the injection. Then by merely reversing the stopcocks (D_3 and D_4) the dogs can be interchanged, or in other words, the dog which was recording its blood-pressure on the smoked drum will pulsate against the mercury in the "dummy" manometer, and the one which was previously pulsating against the "dummy" will record its normal blood-pressure upon the smoked drum. After taking a normal tracing several inches in length, stop the drum; then check the former results by injecting this dog with the same preparation given to dog No. 1; again, take tracing five, ten and fifteen minutes after the injection. Repeat operation by injecting, in a similar manner, the other sample into the dogs on the left-hand side of the drum. This will consume about one hour and fifteen minutes. It is then necessary to wait only about fifteen minutes or until the one and a half hours have elapsed since the first injection was given when the entire procedure can be repeated. This is continued until each dog has received three or four injections. The charts are then measured and the average rise in pressure produced by each preparation is taken as its figure of potency.

To assay one sample of ergot in duplicate and one sample of adrenal extract it is necessary to employ only three animals, two on the one end for the ergot and one on the other end for the adrenal extract.

3. **Uterine Method** (*in situ*). *Apparatus Necessary for Experiment.*—Myocardiograph, counterbalance weights, surgical instruments, cannulæ, tank for saline bath, all-glass syringe, and an apparatus arranged for maintaining artificial respiration.

Animals.—Various animals may be employed, including the dog, cat, and rabbit, but cats of medium size give the best results.

Care of Animals.—See page 238.

Preparation of Experiment.[1]—When the intact animal is to be used it is anesthetized with chloretone (0.3 to 0.4 gm. per kilogram of body-weight) given in solution by means of a stomach-tube. A cannula is then placed in the external jugular vein for the injection of the drugs, and a tracheal cannula is inserted to allow artificial respiration, which is kept up during the entire experiment. The animal is then submerged in a 0.9 per cent. saline bath maintained at the constant temperature of 39° C. The further operative process of exposing the uterus is then carried out by an incision along the linea alba from the ensiform cartilage to the symphysis pubes and the two halves of the abdominal wall drawn apart and secured by means of hooks. The bladder and intestines are drawn aside and secured underneath the salt solution to prevent any irritation by exposure to the air and drying. One horn of the exposed uterus is then freed as much as possible from its attachment to the posterior wall of the body-cavity by tearing away the peritoneal attachments. Especially is it advantageous in securing a uterus with much more freedom of movements to detach the ovary by tearing it free from the ligaments binding it to the posterior wall, although care must be used not to injure the blood-supply in these manipulations.

For the purpose of attaching the uterus to the recording apparatus two silk threads, using a fine round needle, are passed through the uterine horn at a distance of about 2 cm. apart. These are attached to the levers of an ordinary myocardiograph.

For recording the movements a light lever is attached to the myocardiograph, and, as the matter of tension is of great importance, a Harvard light muscle lever is made use of on account of its extreme lightness. Since it has been noted that some uteri will react under considerably more tension than others, the amount of tension is varied by the use of counterbalancing weights until a satisfactory result is secured. These conditions are only attained as a result of experiment with each individual organ, and it is frequently necessary

[1] Edmunds and Hale, Bull. No. 76, Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Serv., pp. 28–29.

to make a number of injections of ergot, using different degrees of tension on the uterus before the best conditions for comparative tests are obtained.

Preparation of Solutions.—All preparations to be tested should be freed from the greater part of alcohol by evaporation on a water bath and made up to fluid extract strength with normal saline solution.

Method of Injecting.—Same as under Epinephrin Standardization. (See page 69.)

Actual Standardization.—"When the mechanical requirements for the experiments are fulfilled the specimens of ergot are injected. More satisfactory results are secured by comparing only two preparations, the unknown and the standard, at a time, injecting them alternately at intervals of not less than five or, perhaps better, ten minutes, and increasing or diminishing the doses until such amounts of the preparation are found which will produce contractions of equal intensity. It is by no means easy to accomplish this, and frequently it calls for the exercise of considerable patience, as, for example, it is not an unusual experience to carry on such an experiment for two or three hours and, on account of the irritability of the uterus as shown by spontaneous contractions, to know very little more about the relative strengths of the two specimens at the end than at the beginning of the experiment.

"In a favorable experiment, for example, one being carried out on the uterus of a virgin cat, there are no spontaneous contractions, and after each injection the uterus responds by a single contraction. In such an experiment it is often possible to get very definite results in a short time, the results from one pair of injections being verified by further injections until there can be no doubt in the mind of the operator as to the relative activity of the two preparations. No standard of comparison can be given, however, which will fit all animals—in the one case the relative strengths of the contraction as outlined above may be taken, while in the second it may be necessary to adopt as an end-reaction the smallest amount of each drug which will produce a contraction, while in a third uterus, which may be irritable and show spontaneous movements, it may be necessary to employ as a standard the smallest amount which will clearly influence these movements, as, for example, by a delay in the relaxation.

Thus it will be seen that each animal appears to be a law unto itself, so far as tension is concerned, and also as to the character of the uterine movements, and that the standard which is to be used in comparing specimens of ergot must be a variable one on that account."

Uterine Method (*Isolated*).—Various methods of standardizing ergot have been devised and employed by various workers utilizing

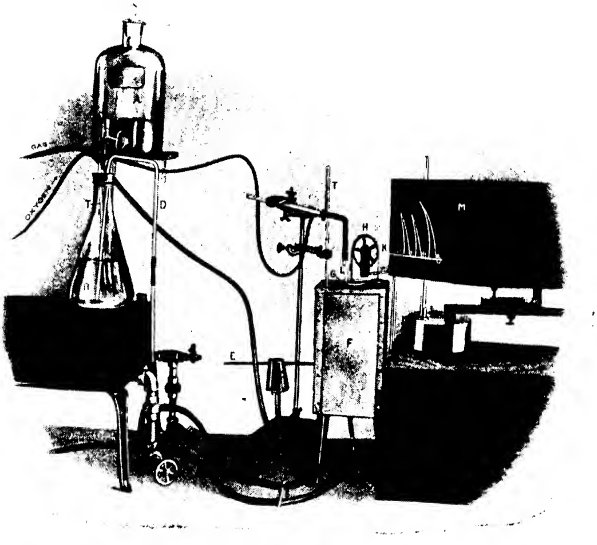

FIG. 32.—Arrangement of a simple apparatus for recording the contractions of an isolated uterus. An improved form of apparatus is shown in Fig. 43.

the fact that this drug has the power of stimulating and thereby increasing the automatic rhythmic contractions of non-striated muscular tissue.

In most cases, however, the muscle was suspended in oxygenated Ringer's solution, contained in a Harvard muscle warmer with a capacity of about 40 c.c. and in practically all cases the Harvard light muscle lever was employed for recording the contractions.

The non-concordant results obtained by these methods have been ascribed to the interference of spontaneous contractions and the increasing irritability of the muscle tissue under the continued influence of the drug. These two factors formed the principal objections to uterine methods.

Animals.—*In view of the above objections to uterine methods pharmacologists, therefore, endeavored to overcome these objections by selecting the uteri of animals manifesting the least degree of normal movements*, preferably those of the cat. But the cat's uterus also proved unsatisfactory. To illustrate, Edmunds and Hale in reporting their observations upon the non-pregnant uteri of cats, state in Bulletin 76, Hygienic Lab. U. S. Pub. Health and Marine Hospital Service: "It is true that the uteri of young cats which may be perfectly quiet in the earlier stage of an experiment after some time may begin to contract spontaneously and increase the difficulty of making comparisons of the effects from successive injections of the drug." In such cases the author states, "It may be necessary to employ as a standard the smallest amount which will clearly influence these movements, as, for example, by delay in the relaxation." (See page 95.)

Later investigations[1] show that the uteri best adapted to standardization purposes, on the contrary, are those which manifest a *high* degree of normal spontaneous movements, preferably those of a non-pregnant guinea-pig, weighing between 275 and 325 gm. Instead of employing a Harvard light muscle lever the free end of the uterus is attached by means of a silk thread to one side of an escapement wheel, to the other side of which is suspended a counterpiose bucket for holding shot. By adding the proper amount of shot to this bucket the operator is enabled to weight the uterus down and thus reduce the amplitude of these movements so they can be controlled. (See Fig. 34 page 95.) Thus the marked spontaneous contractions can be reduced until the uterus is just able to contract

[1] "A New Uterus-contracting Method of Testing Ergot, with comparison with the blood-pressure method," by Paul S. Pittenger and Chas. E. Vanderkleed. Read at the Sixty-first Annual Convention of the Amer. Pharm. Assoc. held at Nashville, Tenn., Aug., 18–25, 1913. Jour. A. Ph. A., July 1914, p. 925.

"The Application of some Muscular Tissues Adapted to Physiologic Standardization," by F. E. Stewart and P. S. Pittenger, Monthly Cyclopedia and Medical Bulletin, Sept., 1913.

under the increased load, or in other words, shot is added until the maximum amount of work that the uterus is normally capable of performing is counterbalanced. Any increase in the amplitude of the contraction after the addition of a given drug can now be produced only by that drug.

Use only healthy guinea pigs weighing between 175 Gm. and 350 Gm. They should not have been pregnant and should not be in heat. It is recommended that young female pigs be segregated at the time of weaning and kept thereafter out of sight and smell of the males.

Care of Animals.—See page 251.

Apparatus Necessary for Experiment. Figures 32 and 33 show the arrangement of a simple apparatus for testing Ergot or Solution Pituitary upon the isolated uterus. A description of this apparatus is given below. A description of an improved but slightly more complex apparatus is described under the standardization of Solution Pituitary (see Fig. 43, page 110).

The uterus is suspended in about 250 c.c. of Locke-Ringer's solution contained in a cylindrical glass vessel (*G*) the lower end of which is plugged with a rubber stopper (*O*) having a central bore. Through the latter passes one arm of a wide glass "T" tube (*J*) which ends flush with the upper surface of the stopper, so that the cylindrical vessel may be completely emptied. This tube passes through a second rubber stopper (*L*) which fills an opening in the bottom of an outer metallic vessel (*F*) which forms a constant temperature water jacket. The temperature of the water in this jacket is kept constant by means of a metallic rod (*E*) which penetrates the wall of the jacket and passes through the water and is soldered to the opposite side of the jacket. The portion of the rod external to the jacket is heated by a protected Bunsen burner (*C*) which slides on the rod. The temperature is regulated by sliding this burner backward and forward until that point is found where the amount of heat transmitted by the rod to the water inside is sufficient to keep the thermometer (*T*) suspended in the water at the proper degree (38° to 39° C.). One of the other arms of the "T" tube is connected by a rubber junction (*X*) armed with spring clamps (*S*) to a waste pipe, by which the cylindrical glass vessel may be emptied.

The remaining arm is connected by a siphon tube (*D*) to a flask (*B*) which holds a small amount of Locke-Ringer's solution for refilling the cylindrical vessel. This flask is kept at a temperature between 40° C. and 45° C. by means of a steam bath (*Z*).

The main supply of Locke-Ringer's solution is contained in a large aspirator bottle (*A*) connected with the small flask by a rubber

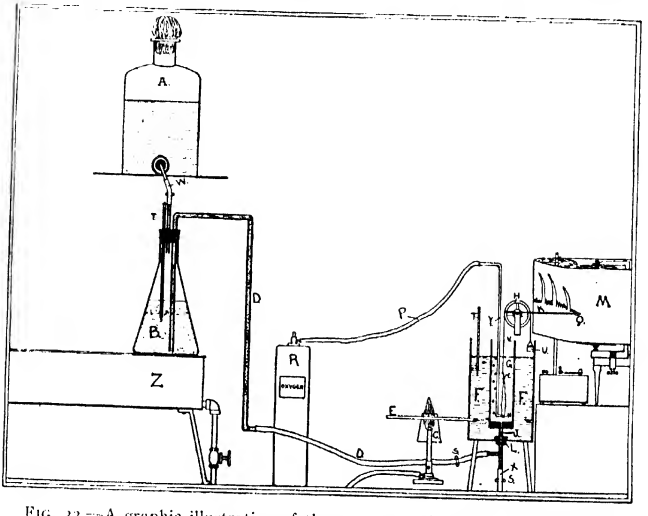

FIG. 33.—A graphic illustration of the apparatus shown in Fig. 32 and will serve to illustrate the following detailed outline of the method. Note that a flat surface is secured at the recording end of the long paper kymograph by the use of an extra single drum kymograph. This is essential in order to record the long, sweeping curves produced by the contraction of the guinea-pig's uterus.

tube (*W*), the object being to avoid exposing the reserve solution to prolonged heat. Heat causes Locke-Ringer's solution gradually to decompose and lose CO_2. The Locke-Ringer's solution in the small flask should be reduced to 39° C. immediately before admitting it to the cylindrical vessel by allowing sufficient cold solution to run into it from the aspirator bottle.

Into the cylindrical vessel containing the Locke-Ringer's solution dips a narrow glass tube (*T*). This tube is turned at a right angle

about half an inch from its lower end. Into this end is sealed a platinum pin (*N*) for attaching the *lower end of the isolated uterus*. The upper end of the tube is connected by means of rubber tubing, (*P*) to an oxygen reservoir (*R*). A constant stream of oxygen is allowed to bubble through a small vent situated at the lower bend of the tube, thus preserving the muscular irritability of the uterus and at the same time stirring the Locke-Ringer's solution.

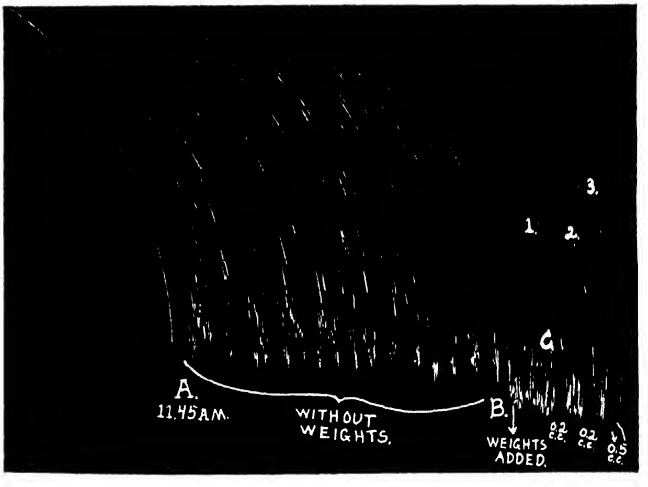

FIG. 34.—Demonstrates first, a very sensitive normally acting uterus (*A* to *B*); second, the action when weighted down by shot (*B* to *C*); and third, the action of ergot or pituitary on the isolated uterus when loaded and working against resistance (1, 2 and 3).

The *other end of the uterus* is fastened to a small platinum hook (*I*) connected to a silk thread (*V*) which passes over an escapement wheel (*H*) and is attached to a pin on the opposite side of the wheel. A counterpoise bucket for holding shot (*U*) is attached to the other side of the wheel. To this wheel is soldered a stylet of aluminum (*K*), the axle of the wheel serving as a fulcrum. To the end of this stylet a pen point is fixed (*Q*) for recording the contractions of the uterus on the revolving drum of the kymograph.

Preparation of Experiment.—Same as under "Isolated Uterus Method of Standardizing Solution Pituitary." (See page 106.)

Preparation of Solutions.—Locke's solution should be prepared according to the directions given on page 330. The samples to be

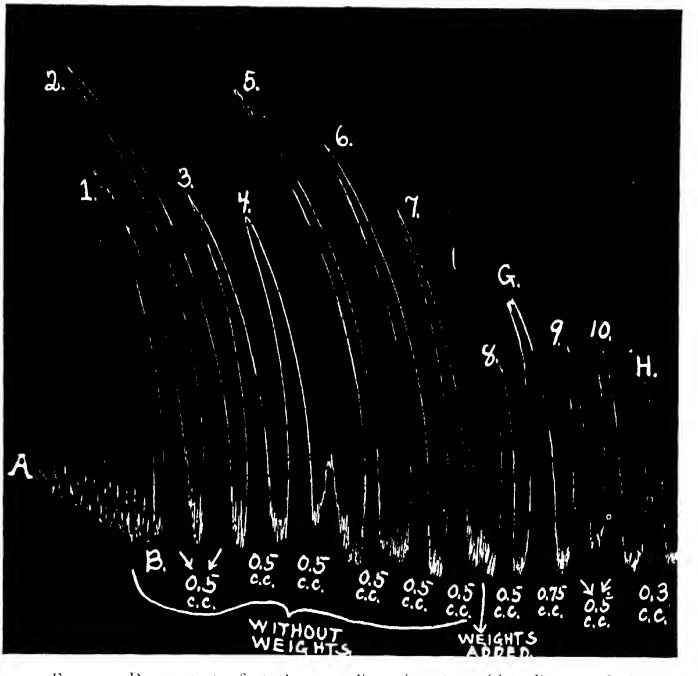

FIG. 35.—Demonstrates first, the normally acting uterus (*A* to *B*); second, the *non-concordant* results produced by repeated doses of the same amount of fluid extract ergot (1, 2, 3, 4, 5, 6, and 7); third, the *concordant results obtained after the uterine contractions are controlled by weights* (8, 9, and 10). The curve *G* and *H* indicate the quantitative results obtained by a larger and a smaller dose.

tested are first freed from alcohol by evaporation on a water bath and then made up to their original volume with water. Solid and powdered extracts should be carefully diluted until 1 c.c. of the dilution represents 1 gm. of drug.

Standard.—Although this method is not official for testing ergot or its preparations the "Official Standard Fluidextract" may be employed as a basis of comparison.

Actual Standardization.—After all preliminary arrangements have been completed a small dose (0.3 0.5 c.c.) of the standard preparation is now pipetted into the Locke-Ringer's solution in which the uterus is suspended. If all conditions are ideal the uterus which was recording small rhythmic contractions will now forcibly contract and record its contraction by a long sweeping curve. After the curve reaches its maximum and commences to decline (which may require from five to fifteen minutes) the medicated Locke-Ringer's solution is quickly run off and replaced by fresh solution, previously adjusted to the proper temperature. The momentary exposure to the air while changing the solution generally causes the uterus to contract rather forcibly, thus markedly increasing the amplitude of the curve produced by the action of the drug. It is necessary, therefore, in changing solutions to hold the escapement wheel for a few seconds or until the uterus is again covered with the saline solution. This will prevent the record from being interrupted by contractions not produced by the drug. The curve now quickly returns to normal and the uterus continues to record its small rhythmic contractions.

Should the uterus chance to be *very sensitive* a dose of 0.5 c.c. of the standard preparation may produce a contraction so strong that it will carry the writing pen off the smoked chart. In such cases it is necessary to reduce the dose. If, however, the uterus still continues to give such marked contractions shot should be added until the contraction can be controlled. On the other hand, should a dose of 0.5 c.c. not produce contraction, the dose should be increased to 1 or 1.5 c.c. If, however, doses of 1.5 c.c. do not call out contractions, shot should be removed until a marked contraction is produced by these doses.

After thus adjusting the apparatus two successive doses of equal amounts of the standard solution should be administered. If the resultant contractions are equal the uterus is giving concordant results and is ready for assay purposes. In order to determine the relative activity of an unknown preparation it is now merely neces-

sary to give progressively increasing or decreasing doses of the unknown preparation until that amount is found which will produce contractions of an equal amplitude as those produced by the standard preparation.

FIG. 36.—This chart clearly demonstrates the concordant results from repeating doses of equal amount. Figs. 1, 2, and 11 indicate quantitative results obtained by varying the doses, and demonstrate the accuracy of this method of standardization of ergot.

Description of Charts.—The terms "no weights" and "weight added" used in the descriptions of the charts refer to the shot used in inhibiting the normal contraction of the uterus, not to the counterpoise employed to keep the uterus suspended in the Locke-Ringer's solution.

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CHAPTER VII

THE PITUITARY BODY

The role which the pituitary body or hypophysis plays in life has until recently been a mystery. It was at first thought that its function was to lubricate the nasal cavities. This belief, however, was soon discarded and replaced by the supposition that the gland was,

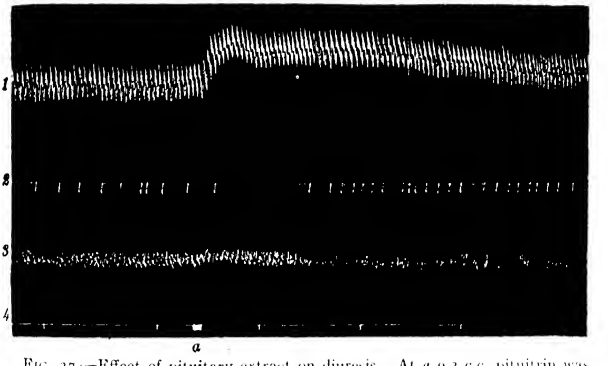

FIG. 37.—Effect of pituitary extract on diuresis. At *a* 0.3 c.c. pituitrin was injected intravenously ("repeat dose"). 1, Carotid blood-pressure; 2, urine registered by drops; 3, carotid pulse; 4, time, one minute. (Hoskins and Means.)

like the appendix, of no use at all. Later, however, it was proven by Vassale and Sacchi,[1] Caselli,[2] and others that the gland plays a very important role and is **absolutely necessary to life.** It has also been found that acromegaly and other diseases are due to functional disturbances produced by an over or an under secretion of this gland

[1] Vassale and Sacchi: Rivista Sperimentale de Freniatria, p. 83, 1894.

[2] Caselli: Studii anatomici e sperimentali sulla Fisiopatologia della Glandola pituitaria, 1900.

and that its removal causes death. According to Sajous[1] the *anterior lobe may prove to be the center of the adrenal system.*

The pituitary body varies in **size** *according to the age and species* of the animal. The gland most commonly used in therapeutics is that obtained from the ox, and is about 3/4 in. in diameter.

The gland is **composed of two parts or lobes**—the anterior and the posterior or infundibular. The smaller or posterior lobe, which forms only about 10 to 15 per cent. of the total gland, is the more important therapeutically. This lobe contains the active blood-pressure raising and uterine-contracting principle and is, therefore, the portion of the gland which is employed in the manufacture of the official "Liquor Pituitari." The anterior lobe contains a blood pressure lowering principle known as "Tethelin" and is the lobe which is so necessary to life.

The total pituitary body contains about 80 per cent. of water, or in other words 100 parts of the fresh gland give about 20 parts of dry substance, containing 2 to 3 parts of the posterior lobe.

Knowledge concerning the chemical composition of the pituitary gland has only recently gained proportions sufficient to warrant the hope that science will ultimately be as successful in isolating and synthesizing its active principle or principles as it has been with the suprarenal gland. Owing to the similarity existing between the physiologic actions of the pituitary and those of the suprarenal gland the theory has been advanced that the active principles of the former will be very similar to epinephrin.

The actions of extracts of posterior pituitary, although somewhat similar to those of epinephrine are different in many ways. It will be noted by a comparison of Figs. 17 and 39 that the rise in blood-pressure produced by pituitary extract is more prolonged than that produced by epinephrine. Pituitary produces a constriction of the coronary vessels and those of the lungs whereas epinephrine has little or no action upon these vessels. The administration of pituitary produces increased cardiac activity which is accelerated after the vagi have been cut or paralyzed with atropine. The acceleration after cutting or paralyzing the vagi does not follow the administration of epinephrine. Also ergotoxin and apocodeine do not abolish

[1] Sajous: Internal Secretions and the Principles of Medicine, vol. 1, p. 216.

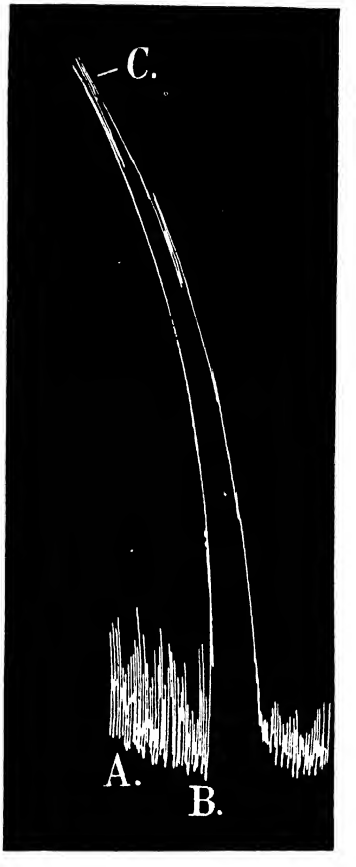

FIG. 38.—Effect of pituitary extract on the isolated uterus.

the effects of pituitary in the manner in which they abolish the effects of epinephrine.

According toMacleod,[1] "The difference in action between the two autocoids is usually explained by assuming that epinephrine acts on the receptor substance associated in some way with terminations of the sympathetic nerve fibres in involuntary muscle, whereas *pituitary acts directly on the involuntary muscle fibres themselves.*"

It has been shown that the activity of the gland can be concentrated into a basic fraction forming salts with acids. It was possible, however, to split this basic fraction into several fractions of different

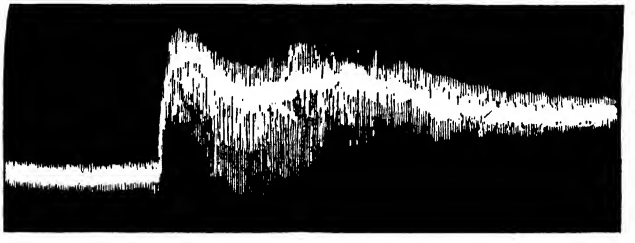

FIG. 39.—Effect of pituitary extract on the blood-pressure.

chemical properties (Führer),[2] which would tend to prove that the action of the pituitary body is due to not one but to the combined actions of several active principles. Schäfer and Vincent[3] had also shown some time before that the blood-pressure-raising principle could be divided into two fractions by their different solubilities in alcohol, one containing a pressor and the other a depressor action.

Abel,[4] however, has shown that the fall in pressure which sometimes occurs after an injection of pituitary extract is not an action of the pituitary hormones but is due to the presence of impurities, particularly histamine. The extensive researches of Abel and his

[1] Macleod: "Textbook of Physiology and Biochemistry" 1926.

[2] Führer: Deutsche medizinische Wochenschrift, March, 1913, p. 491.

[3] Schäfer and Vincent: Journal of Physiology, May 11, 1899.

[4] Abel, J. J.: Harvey Lecture, 1923–24.

associates[1] would tend to prove that the oxytocic, pressor, diuretic and respiratory actions are properties of one and the same substance. Dudley,[2] however, obtained extracts in which the properties were not parallel. The broncho-constriction and depressor actions are apparently due to histamine or a derivative which can be isolated quantitatively from the substance which produces the oxytocic, pressor, diuretic action. (Abel and Nagayama;[3] Roca.[4])

Physiologic experiments have demonstrated that extracts of this gland are valuable therapeutic agents. Thus Magnus and Schäfer[5] and Schäfer and Herring[6] have shown that it **accelerates diuresis**; Oliver and Schäfer[7] that it is valuable for **raising the blood-pressure by arterial constriction** (see Fig. 39); Dale,[8] Bell and Hick,[9] and v. Fränkl Hochwart and Fröhlich[10] that it **excites marked uterine contractions** (see Fig. 38), Ott and Scott[11] that it possesses a rather **marked galactagogue action.**

Standardization of Preparations of Posterior Pituitary

The incomplete knowledge of the chemical composition of the gland and extracts of the same renders it impossible to ascertain by chemical means the comparative value of two or more extracts or fractions of the posterior lobe. We are therefore compelled to resort to physiologic assay methods. Of the various physiologic actions of the gland above mentioned there are three, which present themselves as possible means of biologic standardization, *i.e.*, the action on the blood-pressure, the uterus, and the kidneys.

[1] Abel, Rouiller and Geiling, Jour. Pharmacol. Exp. Ther. 22, 289, 1923.
[2] Dudley, Jour. Pharmacol. Exp. Ther. 14, 295, 1919.
[3] Abel and Nagayama, Jour. Pharmacol. Exp. Ther. 15, 347, 1920.
[4] Roca, Jour. Pharmacol. Exp. Ther. 18, 1, 1921.
[5] Magnus and Schäfer: Proc. Phys. Soc., p. 11, 1901.
[6] Schäfer and Herring: Phil. Trans., 1906 B.
[7] Oliver and Schäfer: Journ. of Phys., XVIII, p. 277, 1895.
[8] Dale: Biochem. Journ., IV, p. 427, 1909.
[9] Bell and Hick: Brit. Med. Journ., I., p. 777, 1909.
[10] v. Fränkl Hochwart and Fröhlich: Arch f. exp. Pathol. u. Therap., LXIII, p. 347, 1910.
[11] Ott and Scott: Proc. Soc. Exp. Biol., New York, 1910.

BLOOD-PRESSURE METHOD FOR STANDARDIZING POSTERIOR PITUITARY EXTRACTS

Apparatus Necessary for Experiment, Animals, Care of Animals, Preparation of Experiment, Method of Injecting.— Same as under "Epinephrine Standardization," see page 64.

Standard.— Same as under "Isolated Uterus Method" (see page 119).

Actual Standardization. After all preliminary arrangements have been completed about 0.2 c.c. of the standard extract should be injected into the femoral vein. If this produces a rise in blood-pressure less than 24 mm. (12 mm. as recorded by the U-shaped manometer) the dose of the standard extract should be increased. If on the other hand the injection of 0.2 c.c. produces a rise of more than 40 mm. the dose should be decreased, as large doses markedly influence the succeeding injections. (See Fig. 40.) After the "standard dose" of the standard solution is thus determined a "standard dose" of the unknown solution is injected and the rise in pressure compared with that produced by the standard solution. If the difference is very great the unknown solution is strengthened or diluted as the case may be. The size of the injection is then increased or decreased until that dose of the unknown solution is found which will cause the same rise in pressure as that produced by the "standard dose" of the standard solution. Occasional injections of the standard solution should be made to show the variation in the reaction of the animal, due to the effects of previous injections. Final equality is secured by injecting alternately the standard and the unknown solutions until the average rise of several consecutive injections is practically equal.

The fact that the blood-pressure method involves the simplest technique together with its satisfactory and almost universal use as a means of standardizing epinephrine and suprarenal extracts would at first lead one to believe that this method would also be the most satisfactory one for standardizing pituitary extracts. It has, however, serious disadvantages in the latter case. As before stated the blood-pressure-raising principle can be divided into two parts, one possessing a pressor and the other a depressor action. Prof. Führer

of Frieberg[1] has also shown that the sum of the basic principles tested by him caused marked uterine contractions and only a slight pressor action, which was almost completely masked by a marked preliminary depressor action.

Furthermore extracts which have been deprived of their depressor action by fractionation with alcohol showed marked pressor effects, while on the other hand, they were sometimes almost entirely free from action on the uterus.

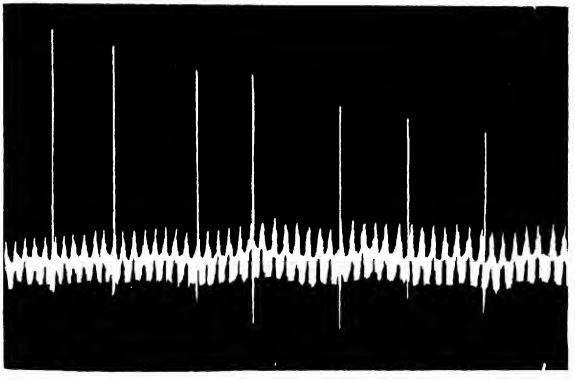

FIG. 40.—Shows the gradual decrease in the rises in blood-pressure produced by repeated injections, of equal size, of pituitary extract.

Another serious drawback to the blood-pressure-raising method is the fact that the active principles of pituitary extract are not nearly as rapidly oxidized as those of the suprarenal gland and therefore repeated injections of equal sizes produce unequal rises, the subsequent ones generally showing a waning of the pressor action and an increasing prominence of the preliminary depressions.

Still another objection to the blood-pressure method is its comparatively low sensitiveness; in other words it requires, in most cases, a rather large variation in the size of the injection to produce a variation in the resultant rise. This latter objection is especially serious when comparing two or more samples for research purposes,

[1] Führer, Deutsche medizinische Wochenschrift, 1913, No. 11, p. 491.

in which case a mistake of 20 to 30 per cent. in interpreting the results of an assay may cause a considerable loss of time. The greater sensitiveness of the uterine method is shown by Figs. 41 and 42, which show the results obtained from tests by both the blood-pressure and isolated uterus methods, in order to determine which was the more active of two pituitary extracts made by different processes. According to the blood-pressure method both extracts showed the same activity while when tested by the uterine method, which is far more sensitive, 0.25 c.c. of one extract proved to be more active than 0.3 c.c. of the other.

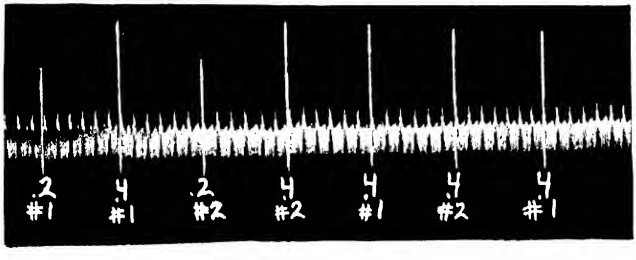

FIG. 41.—Tracing showing equal rises in blood-pressure from equal amounts of the two extracts of pituitary mentioned above.

It has been shown by Dale and Laidlaw that **methods of standardization based upon the diuretic action of pituitary extracts** are also unsatisfactory because of the tolerance produced by the first injection. They state that if small doses are used in order to overcome this tolerance, "it may be difficult to distinguish genuine effects from the spontaneous variations of urinary flow which occur in almost any experiment however constant the controllable conditions."

OFFICIAL ISOLATED UTERUS METHOD FOR STANDARDIZING POSTERIOR PITUITARY EXTRACTS

The uterine methods are based upon the fact that certain drugs have the power of stimulating and thereby increasing the automatic (spontaneous) rhythmic contractions of non-striated muscular tissue.

The action upon the isolated uterus has, in the hands of most workers, proven to be a very satisfactory method of valuating posterior pituitary extracts. By this method a small uterine muscle is suspended in a comparatively large volume of oxygenated Locke-Ringer's solution, which can be readily removed and replaced by a

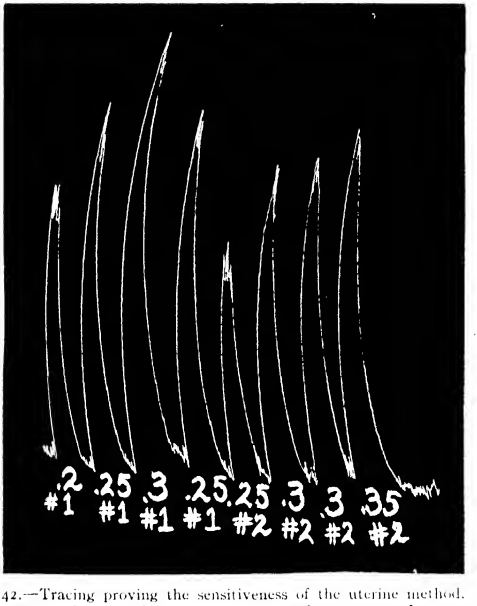

FIG. 42.—Tracing proving the sensitiveness of the uterine method. Chart shows 0.25 c.c. of extract No. 1 to be stronger than 0.3 c.c. of extract No. 2. By the blood-pressure method (owing to its lower sensitiveness) both preparations show the same activity. (See Fig. 41.) (Also see Fig. 56.)

fresh volume. This gives a much better chance, than presented by other methods, for the tissue to recover its original condition by the rapid washing out of the principle or the reduction of its concentration to below the threshold of activity.

Animals and Care of Animals.—Same as under "Isolated Uterus Method for Standardizing Ergot." (See page 92.)

Method of Injecting.—The doses of the standard and unknown solutions are added to the Locke-Ringer's Solution, in which the uterus is suspended in the muscle chamber. The doses should be accurately measured to the hundredth of a c.c. This is best accomplished by means of an all-glass "Tuberculin" syringe.

The muscle chamber should be graduated to 100 c.c. and filled each time with the Locke-Ringer's solution *exactly* to the graduation. After filling the syringe, all air bubbles and excess solution should be expelled leaving the *exact dose* in the syringe. The syringe needle should then be immersed in the Locke-Ringer's solution, in the muscle chamber, and the contents of the syringe expelled. The stream of oxygen bubbling through the solution is sufficient to thoroughly mix the solutions.

Apparatus Necessary for Experiment.—The simple form of apparatus described under the "Isolated Uterus Method for Standardizing Ergot" (see page 91) gives very satisfactory results and was in continuous use for many years. During this time, however, its limitations were carefully studied with the result that several details were noted in which marked improvements could be made.[1,2]

First.—With the apparatus described on page 91 the uterus was subjected to more or less shock at times due to the fact that it was impossible to always have the temperature of the Locke-Ringer's solution in *B* (Fig. 33) at exactly 38° C. the instant it was necessary to use the same for refilling *G*. It was thought advisable, therefore to make the constant temperature bath *F* of sufficient capacity to accommodate the bottle containing the warm Locke-Ringer's solution for refilling *G*. With this stock solution in the same water bath as the cylinder containing the uterus it must necessarily be of the same temperature and thus produce no shock to the uterus when the drugged solution is run off and fresh solution run in.

Second.—The metal tank was replaced by glass which enables the operator to observe to a better advantage the rate at which the

[1] "An Improved Apparatus for Testing the Activity of Drugs on the Isolated Uterus," by Paul S. Pittenger, Jour. A. Ph. A., June, 1918.

[2] "An Improved Apparatus for Testing the Activity of Drugs on the Isolated Uterus," by Paul S. Pittenger, Amer. Jour. Pharm. Sept., 1927.

oxygen is flowing, the amount of solution in stock bottle and the temperature of the bath.

Third.—The brass rod and bunsen burner for heating the water in bath was replaced by an electric immersion heater, the temperature of the water being automatically regulated by means of a toluol-mercury thermostat.

Fourth.—The escapement wheel was improved.

Fifth.—An elevator was added to accommodate two aspirator bottles of water for furnishing air pressure to force the Locke-Ringer's solution from the stock bottle into the cylinder containing the uterus. This has since been replaced by the use of a rubber atomizer bulb.

The complete improved apparatus is shown in Fig. 43. Figure 44 is a graphic drawing showing the arrangement of the stock bottle, thermostat, heater, cylindrical vessel and stirrer, within the constant temperature bath. The labeling of corresponding parts is the same in both figures and will serve to illustrate the following description:

The uterus is suspended in 100 c.c. of Locke-Ringer's Solution contained in a cylindrical tube (1), the lower end of which passes through a rubber stopper (2) which fills an opening in the bottom of the outer glass vessel (3) which, when filled with water, forms a constant temperature bath.

The water in the bath is heated by means of a "Hot Point" electric immersion heater (4). The temperature of the water is automatically kept at 38° C. by means of a toluol-mercury regulator (5) which makes and breaks the circuit to the relay (6) which in turn makes and breaks the electric current (110 or 220 volt) from plug (7) to the heater. The heat is evenly distributed throughout the water by the high speed turbine stirrer (8) which is driven by the motor (9).

The lower end of the cylindrical tube is connected by a rubber junction (10) to one arm of a glass "T" tube (11), one other arm of which is connected by rubber tubing (12) armed with a spring clamp (13) to a waste pipe by which the cylindrical glass tube may be emptied. The remaining arm of the "T" tube is connected by rubber tubing armed with a spring clamp (14) to the lower end of the glass tube (15) which passes through the rubber stopper (16)

which fills the opening in both the bottom of the outer glass vessel and the stock bottle (17), which is filled with unmedicated Locke-Ringer's Solution.

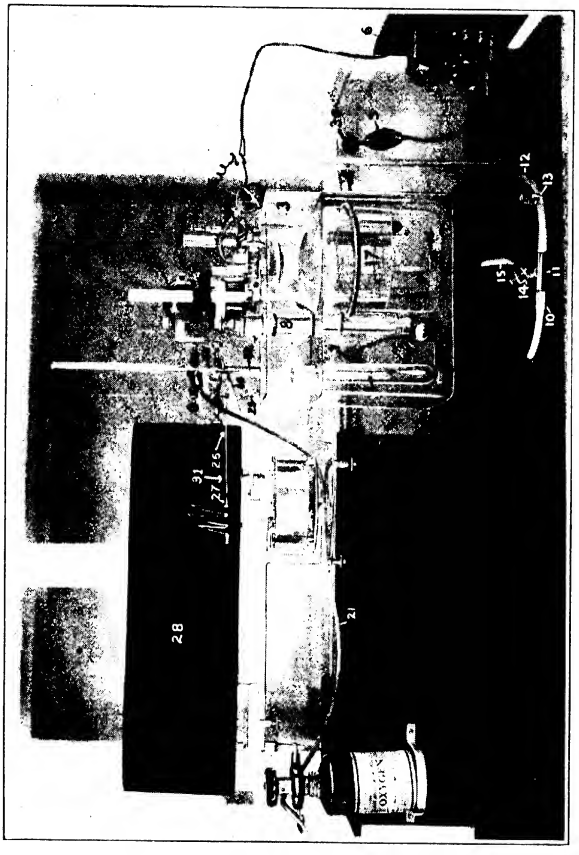

FIG. 43.—Improved apparatus for testing the activity of drugs upon the isolated uterus. (Pharmacologic Research Laboratory, Shark and Dohme.)

Therefore, when the spring clamp (14) is opened the unmedicated solution from the stock bottle (17) is forced through the connections

just described by air pressure, formed by compression of the atomizer bulb (18), into the cylindrical vessel (1) containing the uterus.

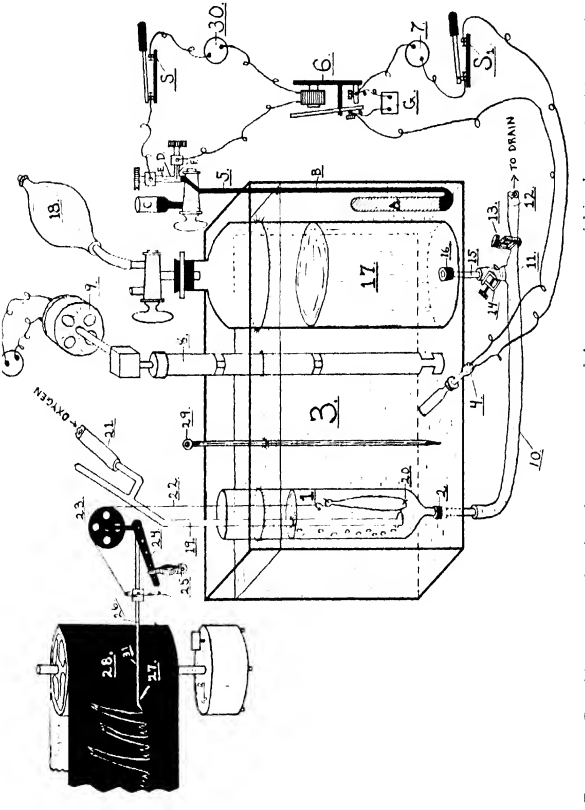

FIG. 44.—Graphic drawing showing the arrangement of the apparatus within the constant temperature bath and method of connecting the thermo-regulator (5) with the relay (6) and the heater (4).

Into the cylindrical vessel containing the Locke-Ringer solution dips a narrow glass tube (19). This tube is turned at a right angle about half an inch from its lower end. Into this end is sealed a

platinum pin (20) for attaching the *lower end of the isolated uterus*. The upper end of the tube is connected by means of rubber tubing, (21) to an oxygen reservoir. A constant stream of oxygen is allowed to bubble through a small vent situated at the lower bend of the tube, thus preserving the muscular irritability of the uterus and at the same time stirring the Locke-Ringer solution.

The *other end of the uterus* is fastened by means of a small hook connected to a silk thread (22) which passes over the wheel of a "Becker" universal lever (23). The weight of the counterpoise lever (24) is increased by attaching an artery clip (25). The tension upon the silk thread may be increased or decreased both by changing the position of the counterpoise lever, which may be moved on its fulcrum and by changing the position of the artery clip on the lever.

The end of the writing lever (26) is attached to one end of a straw stylet (31). To the other end of the straw a celluloid writing point (27) is fixed for recording the contractions of the uterus on the revolving drum of the kymograph (28).

A thermometer (29) is placed in water bath in order to determine whether the toluol-mercury regulator in maintaining the proper temperature.

It often happens that after suspending a uterus in the oxygenated Locke-Ringer's solution it will relax to a greater extent than was expected by the operator and the writing point will fall below the smoked paper. Occasionally a uterus will make apparently normal contractions and react readily to the drug, but after two or three doses will suddenly relax to a much greater extent and establish a new normal far below the original. It is not convenient to lower the paper on the double drum kymograph to bring the writing point into proper position. Likewise, it is sometimes impossible to lower the glass tube (19) in order to raise the writing point. In order to make this possible without moving the kymograph or the glass support, the thread (22) is tied into a series of small loops about 1/8 inch apart. These loops are attached to the writing lever by placing them over a small hook which is soldered to the writing lever at the point where the straw is attached. The writing point is raised to the desired position and the small hook placed through the nearest loop.

Figure 45 shows the construction of the toluol-mercury thermostat. That portion of the glass tube which is lightly shaded (see Fig. 47) represents toluol (*A*) and the black portion (*B*) represents mercury. In order to adjust the thermostat so that it will throw the relay at 38° C. the two reservoirs *C* and *D* are filled with mercury, the stop-cock placed in the position shown and the bulb *A* placed in a water bath. The temperature of the bath is increased until the surface of the mercury just touches the platinum contact wire *E*. The stop-cock is then turned to the opposite position (connecting *C* and *B*) and the temperature of the bath adjusted to exactly 38° C. At exactly 38° C. the stop-cock is again turned to the original position. The thermostat will then be adjusted so it will automatically "make" the current flowing to the relay through the platinum contacts *F* and *E*.

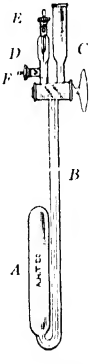

FIG. 45.—Toluol-Mercury Thermostat.

The platinum contact point *F* is connected electrically with the one side of the coil of the electro-magnet of the relay while the contact point *E* is connected to the one side of the plug (30). The other side of the plug is connected with the other side of the coil of the relay magnet.

Before connecting the coil of a relay directly with a 110 volt circuit as described above care should be taken to see that the relay is of the proper type. Many of the stock relays although made to make and break a 110 or 220 volt circuit are equipped with operating coils made to be operated by a 6 volt circuit. A 110 volt circuit would burn out such a relay. With a relay of this type the coil should be connected with a storage battery or transformer.

With a toluol-mercury thermostat as described it is not advisable to pass the regular 110 volt lighting circuit through the mercury. The relay used in this apparatus, therefore, is a stock Cutler-Hammer Relay #232 (with the contacts normally closed) which has the coil so constructed that it is satisfactory for continuous duty and may be connected direct to the 110 volt circuit. Although the voltage remains the same, the coil reduces the amperage sufficiently to allow the use of the mercury thermostat.

The relay must be of the type which breaks the contact in the circuit to the heater when the magnet is active and closes the circuit when the magnet is inactive. In other words, when the mercury in the thermostat closes the circuit to the coil of the relay the relay must break the circuit to the heater and vice versa.

The switches (S and S_1) are to break the circuits when the thermostat is not in use.

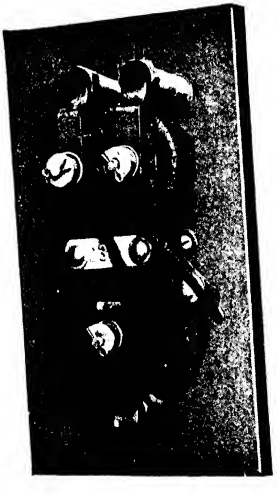

FIG. 46.—Cutler-Hammer Relay.

In order to automatically maintain the temperature of the bath at 38° C. arrange the apparatus as shown in Fig. 44; regulate thermostat as already outlined; start stirrer and close switches. The heater will then produce a gradual increase in the temperature of the water which will cause the toluol in the thermostat to expand and the mercury to rise. When the temperature of the water reaches exactly 38° C. the mercury touches the contact points F and E which completes the battery circuit and allows the current from the batter-

ies (or transformer) to flow through the coil of the relay, thus forming an electro-magnet which lifts the armature of the relay and in turn breaks the contact which stops the current flowing to the heater.

With the gradual lowering of the temperature of the water the toluol contacts and causes the mercury to fall away from the platinum contact E, thus breaking the battery current which is passing through the coil of the relay. This break allows the armature of

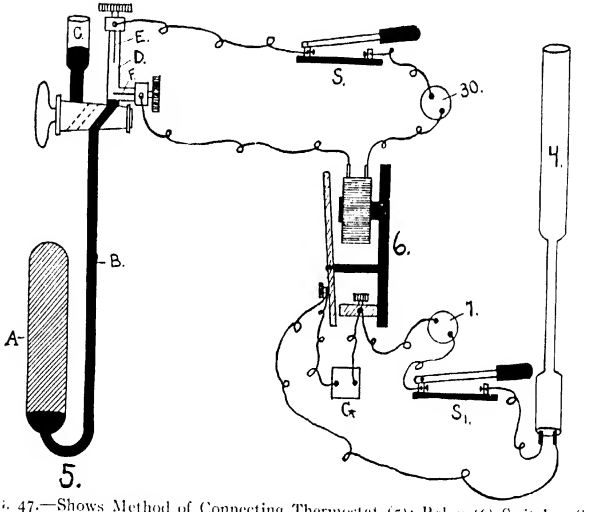

FIG. 47.—Shows Method of Connecting Thermostat (5); Relay (6) Switches *S* and S_1 and Heater (4) with Electric Plugs (7 and 30).

the relay to fall away from the magnet and make the contact which again allows the current to flow to the heater. The heater gradually increases the temperature of the water until at 38° C. the mercury in the thermostat again makes a contact and the whole operation repeats itself.

An even temperature is maintained throughout the bath by the use of a Cenco high speed turbine stirrer (Fig. 48) which keeps the water in constant circulation without undue agitation.

The accuracy of the Pituitary Assay is to a marked degree dependent upon the uniformity of the temperature of the water bath. The U.S.P. states "The temperature of the bath should be between 37 and 38° C. but should not vary more than one-tenth of a degree throughout the whole assay." It is apparent, therefore, that in order to comply with the above strict temperature requirements an automatic temperature control is necessary.

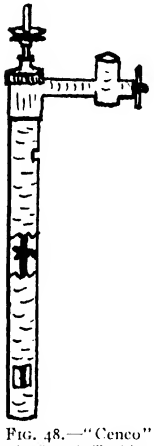

FIG. 48.—"Cenco" High Speed Turbine Stirrer.

Preparation of Experiment.—The animal is bled by quickly severing the carotid artery with a sharp-poitend scissors. The spinal column is then sevcred wtih strong scissors. One horn of the uterus is then quickly excised together with the ovary which is left attached by means of the fold of broad ligament in which the Fallopian tube runs.

Uteri differ greatly in their mutual relation as to power, and muscular structure. (See Fig. 49.) Some specimens are greatly deficient in muscular substance and act feebly while other specimens show greater muscular development and contract strongly. Some specimens prove absolutely inert and will not respond at all. The normal activity, however, practically runs parallel with the amount of muscular tissue present; the "stringy" uteri are all deficient in normal activity and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive. This knowledge enables the operator to save considerable amounts of time as it renders it possible for him to distinguish between active and inactive uteri before connecting them with the apparatus.

The one horn of the uterus is then quickly transferred to the oxygenated Locke-Ringer's solution in the cylindrical vessel and attached to the platinum pin and hook above referred to (the ovary is fastened to the hook suspended from the escapement wheel and the lower end of the horn is fastened to the pin at the lower end of the oxygen tube). The manipulation and exposure followed by the

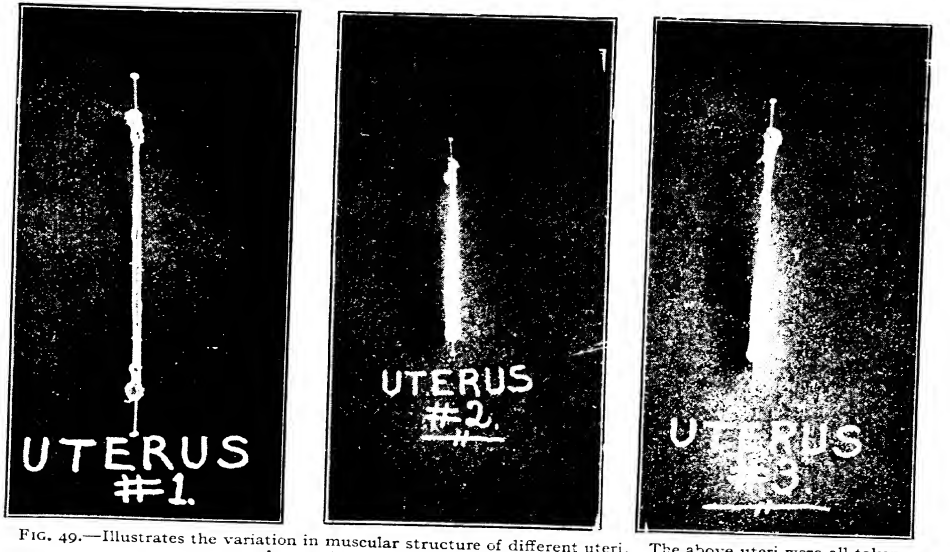

FIG. 49.—Illustrates the variation in muscular structure of different uteri. The above uteri were all taken from guinea-pigs weighing from 280 to 320 gm.

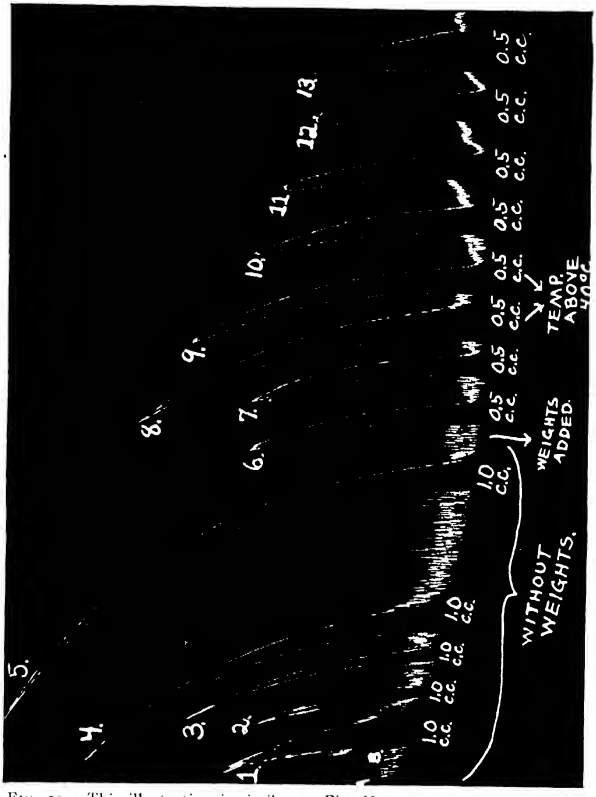

FIG. 50.—This illustration is similar to Fig. No. 35. *A* to *B* show normal contractions; 1, 2, 3, 4, 5, non-concordant results obtained without weights; 6, 7, 10, 11, 12, 13, concordant results obtained after weights were added.[1] The curves 8 and 9 indicate contractions produced under the increased stimulation due to rise in the temperature of the Locke-Ringer's solution, and show the necessity of maintaining an even temperature during the experiment.

[1] With apparatus of the design shown in Fig. 32 weights are added to increase the tension on the uterus. With the apparatus shown in Fig. 43 the tension is increased by changing the position of lever (24) and clip (25).

immersion in the warm solution will almost invariably produce a high degree of tonus, which, however, gradually diminishes until the uterus returns to its normal condition. If at this point the uterus does not exhibit *strong* rhythmic contractions it should be discarded and replaced by a new one. The tension upon the uterus is now increased *until the uterus can make only small rhythmic contractions.* (See page 112.)

Conditions are now suitable for determining the activity of the drug to be tested or standardized.

Necessity for Standard Solution.—Due to the variation in susceptibility of the different uteri (see Fig. 49 page 117). This method must essentially be comparative and not absolute. This necessitates the adoption of a standard with which the activity of the unknown can be compared. The comparison must of course always be made on the same uterus.

The U.S.P. IX adopted as standard a 1 to 20,000,000 solution of beta-iminazolyl-ethylamine-hydrochloride. Shortly after the publication of the U.S.P. IX, however, the author published the results of experiments[1] which showed that the standard substance adopted by the revision committee was unsatisfactory due to the fact that it deteriorated quite rapidly and that the standard adopted was very low because by comparison it was found that the commercial preparations on the market were from three to five times as active as the adopted standard. The findings of the author were corroborated by Eckler[2] and Hamilton.[3]

During the period that the U.S.P. IX was official many individuals and various association committees carried out cooperative experiments in an endeavor to obtain a satisfactory standard. This work resulted in the selection and inclusion of the following standard in the U.S.P. X.

Preparation of Standard Solution.—Carefully weigh a suitable amount of the dried Standard Powdered Pituitary, place this weighed powder in a small agate mortar and moisten with a few drops of distilled water containing 0.25 per cent.

[1] "Preliminary note on the Value of Beta-Iminazolyl ethylamine-hydrochloride as a Standard for Testing Pituitary Extracts" by Paul S. Pittenger and Charles E. Vanderkleed, Jour. A. Ph. A., Feb. 1917, 131.

[2] Eckler, Amer. Jour. of Pharmacy, May, 1917, p. 195.

[3] Hamilton, Amer. Jour. of Pharmacy, Feb., 1917.

of acetic acid. Triturate the moistened powder thoroughly until the whole is of an impalpable frothy consistence. Add a few c.c. of the 0.25 per cent. acetic acid solution and stir the mixture thoroughly. Transfer to a hard glass test tube or beaker; rinse the small agate mortar with the acetic acid solution and add the rinsings to the pituitary mixture; then add enough 0.25 per cent. acetic acid to make the final volume of the mixture of the same number of c.c. as the number of milligrams of dried pituitary powder originally taken. Heat this mixture to the boiling point for not more than one minute and filter. The filtrate contains in each c.c. the active principle of 0.001 gm. of the dried standard powder. Place this solution in hard glass ampuls and sterilize by fractional sterilization for twenty minutes on three successive days at a temperature not exceeding 100° C. Preserve in a cool place (from 5° to 20° C.). This standard solution should not be kept for more than six months.

Standard Powdered Pituitary.—The U.S.P. directs that the Standard Powdered Pituitary supplied by the Bureau of Chemistry, U. S. Department of Agriculture should be prepared as follows: Select a number, not less than 25, of fresh posterior lobes of the pituitary body of cattle, removed within thirty minutes of the death of the animal and carefully free them from all extraneous tissue, immediately after their removal. As soon as they are so prepared, drop them into a flask containing a quantity of acetone corresponding to not less than 4 c.c. of acetone for each pituitary body. Allow them to remain in the acetone for three hours, and then remove them and immediately cut them into small pieces with scissors and place the material in fresh acetone equal in quantity to that first employed. Leave the material in the acetone over night and then remove it and dry it in a vacuum desiccator over calcium chloride at a temperature not above 50° C., for five hours. At the end of this time remove the material and grind it in a mortar until it will pass through a #40 sieve. Dry this powder for at least 12 hours in a vacuum desiccator over calcium chloride at a temperature not over 50° C. Extract this dried powder in a small Soxhlet continuous extraction apparatus with acetone for three hours and again dry in a desiccator over calcium chloride for twelve hours. This dried powder should be preserved in a cool, dark place, in sealed ampulus *in vacuo*, or in vacuum desiccators over calcium chloride until used for the preparation of the standard solution.

Preparation of Unknown Solution.—In most cases the unknown solution consists of a "commercial" solution of Pituitary Extract which is to be adjusted to standard activity before marketing.

As Desiccated Posterior Pituitary is an article of trade and used in tablets, capsules, etc., it should likewise be standardized. A solution should be made of the desiccated powder according to the directions given above for the "Preparation of Standard Solution" by replacing the Standard Pituitary Powder with the desiccated powder of unknown activity. This solution should then be tested

by the above described method, using the solution made from the standard powder as the basis for comparison.

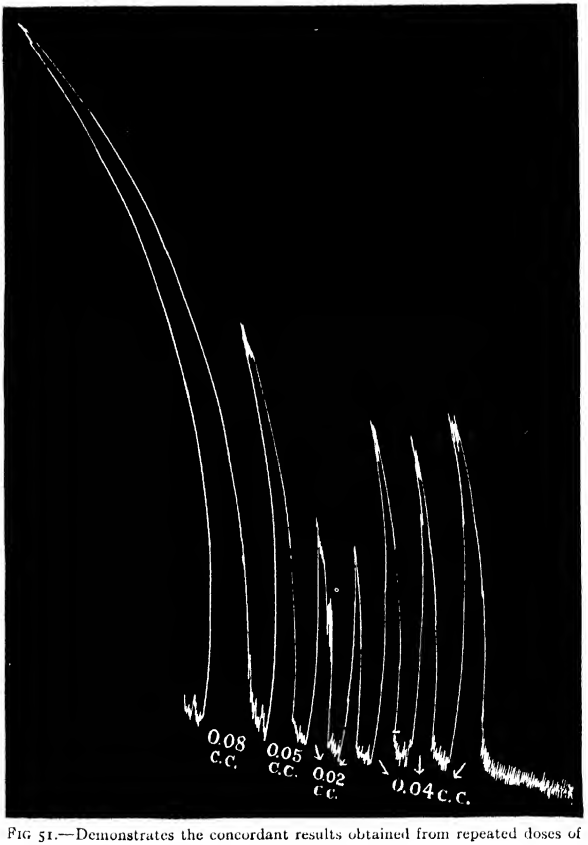

FIG 51.—Demonstrates the concordant results obtained from repeated doses of an equal amount of pituitary extract.

Actual Standardization.—After the uterus has been placed in the apparatus and has attained the condition of uniform low tonus, a

small dose (about 0.1 c.c. of a 1 in 4 dilution) of the standard extract is injected into the 100 c.c. of Locke-Ringer's solution in which the uterus is suspended. If all conditions are ideal the uterus which was

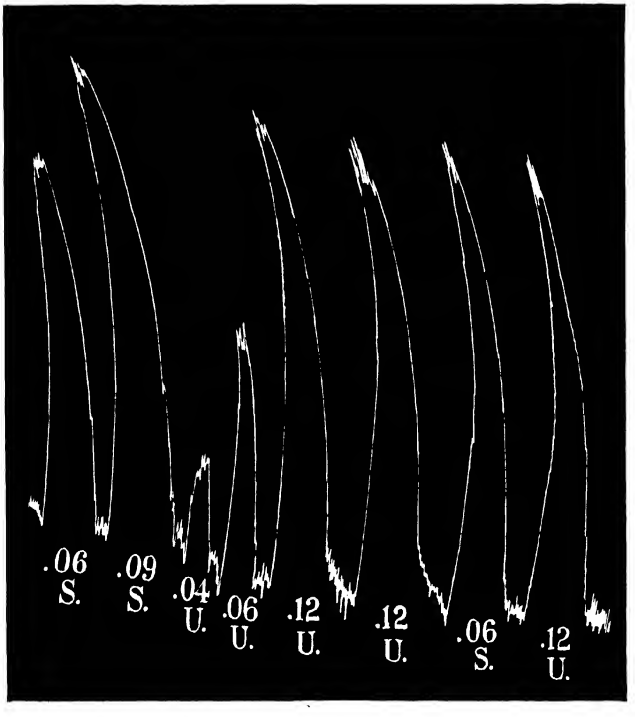

FIG. 52. Tracing showing the results of an assay of pituitary extract on the isolated guinea-pig's uterus. *S*, standard extract; *U*, unknown extract; tracing shows 0.06 c.c. of the standard extract to be as active as 0.12 c.c. of the unknown extract. (Also see Fig. 56.)

recording small rhythmic contractions will now forcibly contract and record its contraction by a long sweeping curve. The dose should be sufficiently large to produce a nearly but not quite maximal tonus. If this initial dose of the standard extract produces a maximal tonus

the drugged solution should be quickly removed and replaced by fresh Locke-Ringer's, after which a smaller dose is tried. If on the other hand, the lever after rising to a position of partial tonus falls again and a wide rhythm ensues, the dose is too small. In such cases the solution should be changed and after a few rhythmic contractions at minimal tonus, larger doses should be tried. If, however, doses of 0.8 to 1.0 c.c. of a 1 in 4 dilution added to the 100 c.c. of Locke-Ringer solution in the muscle chamber, do not produce tonic contractions, the uterus should be discarded and replaced by another.

With a properly working apparatus and a satisfactory uterus, doses of 0.2 to 0.6 c.c. of a 1 in 4 dilution of the "standard" extract should produce suitable submaxinal contractions. That the contraction produced by the injection of a given dose is submaximal, must be proven by the injection of a larger dose which must produce a greater contraction.

After selecting the dose of the "standard" to be used as the basis of comparison and having proven that this dose produces a *submaxinal* contraction the next step is to determine whether or not the uterus is reacting concordantly. In other words, does the uterus respond quantitatively. Doses of equal size must produce equal contractions, as shown by curves of equal amplitude, and larger or smaller doses must produce relatively stronger or weaker contractions. Figure 42 illustrates these points. It will be noted that 0.25 c.c. produces a sub-maxinal contraction. This is proven by the fact that a greater contraction was produced by 0.3 c.c. That the uterus was giving concordant, quantitative results is shown by equal contractions from equal doses and by the fact that the resultant contraction is in direct proportion to the size of the dose.

When a uterus is making *strong normal rhythmic contractions* but does not respond quantitatively to the injections of the drug it will usually be found that the uterus is not under sufficient tension. In such cases a slight increase in the tension, will generally have a tendency to make the contractions more in proportion to the size of the dose injected.

On the other hand when the normal contractions are comparatively small and the usual size doses do not produce strong tonic contractions the tension on the uterus is too great. In such cases

the tension should be decreased until the proper response is obtained. *The accuracy and sensitiveness of the test is almost in direct proportion to the operators experience and ability to properly adjust the tension on the uterus.* After determining the "standard dose" of the standard extract required to produce concordant, submaximal contractions, a "standard dose" of a 1 in 20 dilution of the unknown extract should be given and the resultant contraction compared with that produced by the standard extract. If the difference is *very* great the unknown solution is strengthened or diluted accordingly. The size of the injection is then increased or decreased until that dose of the unknown is found which will cause a contraction of an amplitude equal to that produced by the standard. Occasional injections of the standard solution should be made to insure constancy in the reaction of the uterus. Final equality is secured by injecting alternately the standard and the unknown extract until quantities of the two solutions are found which give equal, submaximal contractions in at least two successive pairs of contractions. The strengths of the two solutions are in inverse ratio to the quantities necessary to produce these equal contractions.

It is not necessarily significant that there occur a slight variation in the amplitude of the curves produced by two successive doses, as a second equal dose of the same preparation will frequently give a contraction of slightly greater amplitude than that produced by the first dose, or may, on the other hand, produce a slightly smaller one. The uniformity of result is more nearly complete with some uteri than others. The uniformity depends to a considerable extent on the *uniformity of interval between the doses*, so that the first dose of a group, either at the beginning of the experiment, or at a later stage following a prolonged interruption, tends to produce a slightly abnormal result in one direction or the other. The succeeding doses, however, generally give concordant results. On the other hand it is inadvisable to give a long series of doses at short intervals as this produces a gradual decline in the sensitiveness of the uterus. It is advisable, therefore, when making a series of comparisons, to give a group of four or five doses at about ten-minute intervals, then allow an interval of about one-half hour or so and then follow with another group, regarding the first member of each group of

curves as probably abnormal. Good uteri will give reliable results for eight to ten hours after being excised and placed in the Locke-Ringer's solution.

This method is by far the best so far proposed, for the standardization of pituitary extracts, as differences of activity which are only just appreciable by the blood-pressure method, under the best conditions, are at once obvious in the test on the uterus without any special care in controlling the regularity of the response.

Official Standard.—**Solution of pituitary,** contains the water-soluble principle or principles from the fresh posterior lobe of the pituitary body of cattle, 1 c.c. having an activity upon the isolated uterus of the virgin guinea pig, corresponding to not less than 80 per cent. and not more than 120 per cent. of that produced by 0.005 gm. of the Standard Powdered Pituitary, prepared as directed below. (See "Preparation of Standard Solution" p. 110.) The solution should be sterile.

In other words Pituitary Solution to be of U.S.P. standard strength must be five times as active as the "Standard Solution."

Unofficial Standard. **Desiccated Posterior Pituitary.**—Although there is no official standard for this product experience has shown that carefully dissected and properly desiccated posterior pituitary lobes possess an activity of 50 per cent. of that of the official "standard pituitary powder."

Notes on Methods of Eliminating Some Difficulties Encountered with Isolated Uterus Method[1]

Experience has proved that satisfactory results cannot be obtained with this method unless all conditions are ideal.

The presence of minute amounts of impurities in the distilled water or chemicals used in preparing the Locke-Ringer's solution will destroy the sensitiveness of the uterus. Bacterial contamination of the Locke-Ringer's solution will poison the uterus and make it impossible to obtain concordant results.

[1] "Isolated Uterus Assay for Pituitary Extract (Notes on Methods of Eliminating Some Difficulties Encountered with the above Method)," by Paul S. Pittenger and Arnold Quici, Jour. Amer. Pharmacentical Assoc. Jan. *1923, 14.*

Variations in the temperature of the solution and the amount of muscular tissue present in the uterus are also factors of prime importance.

The many criticisms of the isolated uterus method to the effect that it gave unsatisfactory results, without stating in what way the method was unsatisfactory, or suggesting means of improving it, led the author to the opinion that in many cases these unsatisfactory results were due to the operator's *failure to observe* some of the essential details which we have found to be the key to success or failure.

The literature does not contain reports showing the minute details and care which must be exercised in preparing the Locke-Ringer's solution, or reports attributing the cause of unsatisfactory results to the Locke-Ringer's solution.

When we stop to consider the fact that when the uterus is taken from the animal's body and placed in the Locke-Ringer's solution, this solution is actually replacing the blood of the animal, it is easy to realize how a slight variation in its composition can markedly influence the results obtained.

DISTILLED WATER

After obtaining satisfactory results for a period of over two years we had a series of 8 or 10 consecutive assays in which it was practically impossible to obtain concordant results.

The trouble was finally traced to *distilled water*. Our source of supply had been changed and upon testing we found that the water was not quite as pure as that previously employed. On returning to the use of distilled water from the original source the trouble was entirely eliminated.

Therefore absolutely pure distilled water is essential. Glass distilled water is to be preferred.

BACTERIAL CONTAMINATION

On one other occasion unsatisfactory results were found to be due to excessive contamination of the Locke-Ringer's solution.

A bulk container had been refilled many times without being completely emptied or washed.

After boiling out all containers, tubing, etc., and filling with new Locke solution, satisfactory results were again obtained.

Our experience has since shown that it is not necessary to actually sterilize the apparatus or Locke-Ringer's solution but it is absolutely necessary that all containers, tubing, etc., be thoroughly cleaned with hot water at least once a week.

SELECTION OF TEST MUSCLE

As stated under "Standardization of Posterior Pituitary" uteri differ greatly in their mutual relation as to power and muscular structure. Some specimens are greatly deficient in muscular substance and act feebly, while other specimens show greater muscular development and contract strongly. (See Fig. 49 p. 117.) Some specimens prove absolutely inert and will not respond at all. The normal activity, however, practically runs parallel with the amount of muscular tissue present; the "stringy" uteri are all deficient in normal activity and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive.

PURITY OF CHEMICALS

When preparing Locke-Ringer's solution only the *highest purity* "Reagent" or "Analyzed Chemicals" should be employed.

By carefully observing the above precautions the author has for many years obtained highly satisfactory and concordant results.

On one occasion, however, we encountered a new difficulty: the uterus after contracting from an initial dose of the extract would not relax upon replacing the drugged solution with fresh Locke-Ringer's solution.

A series of experiments proved that this condition was produced by some variation in the composition of the Locke-Ringer's solution.

Figure 53 shows a tracing of the contraction produced by pituitary extract when solution of the proper composition is employed. *A* to *B* shows the normal contraction. At *B* 0.01 c.c. of pituitary extract was added. At *C* the drugged solution was replaced by plain Locke-Ringer's solution. You will note that under normal conditions the uterus relaxes immediately.

Figures 54 and 55 show tracings of the contractions produced by pituitary extract when the Locke-Ringer's solution is *not* of the proper composition. *A* to *B* shows the normal contraction. At *B* 0.01 c.c. of pituitary extract was added. At *C* the drugged solution was replaced by plain Locke-Ringer's solution.

You will note that in both cases the *uterus failed to relax due to the improper composition of the Locke-Ringer's solution.*

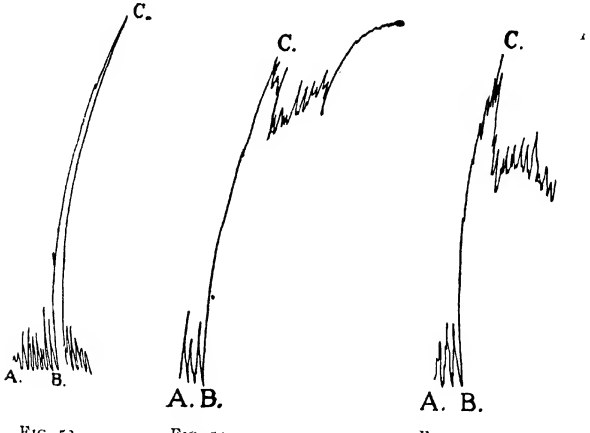

FIG. 53. FIG. 54. FIG. 55.

FIG. 53 shows a tracing of the contraction produced by pituitary ext. when solution of the proper composition is employed. *A* to *B*, normal. At *B*, 0.01 c.c. pituitary ext. added. At *C*, drug solution replaced by Locke-Ringer's solution.

FIGS. 54 and 55 show the contractions produced by pituitary extract when solution of improper composition is employed.

By the process of elimination we found that the *trouble was caused by variations in the sodium chloride employed in making the solution.*

We had always used "Analyzed" sodium chloride and never before experienced any difficulty.

A careful study of the statements on the labels showed that it was absolutely impossible to make any deductions from this information as to why some of the samples were satisfactory and others were not.

We observed, however, that all of the satisfactory lots were in the form of *large* crystals, while all of the unsatisfactory lots were powdered or in small crystals.

We therefore purchased additional lots from different sources and found that in every case the *large* crystals were satisfactory while the salts in the form of powder or small crystals were not.

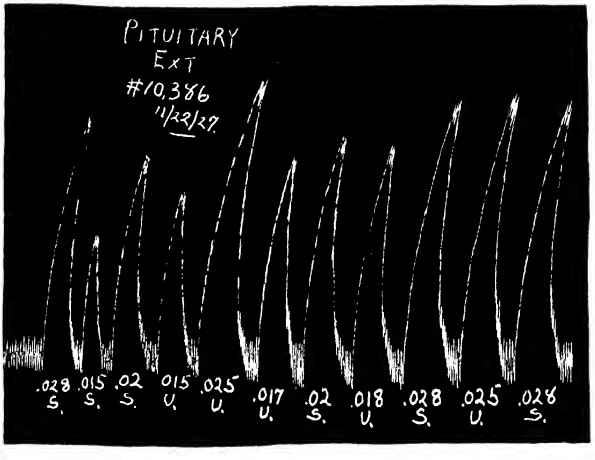

FIG. 56.—Shows tracing of a pituitary assay carried out under the proper conditions. You will note that in the above assay the uterus reacted quantitatively to variations in the dose of 0.002 c.c.

This led us to believe that perhaps the manufacturers take the sample for assay from the "bulk lot" and that powdering is a subsequent operation during which some form of contamination occurs.

Further investigation, however, proved that this is not the case and that there exists an actual difference in the purity or composition of the two forms of salt. The large crystals are from the "first crop" and usually prepared from sodium bicarbonate while the powder or small crystals are from "subsequent crops" and in some cases prepared from other salts.

These minute differences in composition are negligible when the salt is used for ordinary analytical purposes. When used for preparing Locke solution for isolated uterus experiments, however, they are of the utmost importance and the key to success or failure.

Therefore, only "Analyzed" or "Reagent" sodium chloride in the form of *large* crystals should be used in preparing Locke solution.

The uniformly concordant results which we obtain with the Isolated Uterus Method for testing pituitary extracts led the authors to believe that many of the unsatisfactory results reported have no doubt been due to one or more of the factors mentioned above.

Conclusions

In order to obtain satisfactory results with the isolated uterus method of assaying pituitary extracts:

1. Glass distilled water must be used.

2. All chemicals employed in making Locke-Ringer's solution must be of the highest ("Reagent") purity.

3. All apparatus and solution containers must be frequently washed with boiling water, and Locke-Ringer's solution should be freshly prepared.

4. Thin, "stringy" uteri should not be used as they are all deficient in normal activity, and in response to stimuli, while the thick, more muscular uteri are practically all active and sensitive.

5. Practically all of the best grades of sodium chloride on the market in the form of powder or small crystals are unsatisfactory for preparing Locke-Ringer's solution.

6. Only "Reagent" or "Analyzed" sodium chloride in the form of *large crystals* is satisfactory for isolated uterus experiments. Baker's Analyzed *Special Crystals* apparently give the best results.

7. When the above precautions are observed, the Isolated Uterus Method gives better results than any other method so far proposed.

The Effect of Alcohol on the Activity of Pituitary Extract

Many physicians have formed the opinion that the small amount of alcohol used in some cases, to sterilize the hypodermic syringe destroys the action of pituitary extract. In many cases the failure

to obtain the desired results from an injection of liquor pituitari has been attributed to the small amount of alcohol adhering to the inside of the syringe. The author, however, has shown that *small amounts of alcohol do not destroy the physiologic activity of Pituitary Extract.*[1] (See Figs. 57 and 58.)

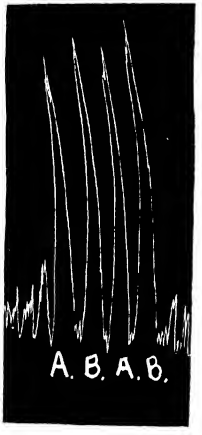

FIG. 57.

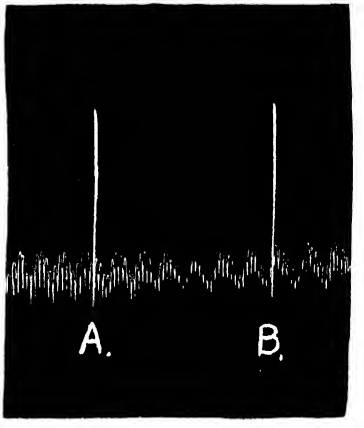

FIG. 58.

FIG. 57.—Shows that small amounts of alcohol do not influence the activity of liquor pituitari upon the isolated uterus. *A*,—0.025 c.c. liquor hypophysis. *B*,—0.025 c.c. liquor hypophysis to which has been added 0.05 c.c. alcohol.

FIG. 58.—Shows that small amounts of alcohol do not influence the effects of liquor pituitari upon the blood pressure. *A*,—0.2 c.c. liquor hypophysis. *B*,—0.2 c.c. liquor hypophysis to which has been added 0.05 c.c. alcohol.

METHOD OF TESTING PREPARATIONS OF ANTERIOR PITUITARY

Although preparations of the posterior lobe are used to a much greater extent than those made from the anterior lobe, the value of the latter is being more and more appreciated, especially by those who treat mental and physical mal-development in children.

[1] "The Effect of Alcohol on the Activity of Liquor Hypophysis," by Paul S. Pittenger, Jour. Amer. Pharmaceutical Assn. Oct. 1918, 851.

The anterior lobe is a definite gland of internal reaction and is supposed to control skeletal and other structures of the growing animal.

As before stated the anterior lobe is necessary to life and cannot be removed without causing death. Its partial removal or disease brings about a condition of adiposity, asexualism and reduced bodily activities. Its increased use in therapeutics makes it desirable to test the activity of the various commercial preparations of this gland.

Blood Pressure Method

Apparatus Necessary for Experiment, Method of Injecting.—Same as under "Blood-pressure Method for the Standardization of Epinephrine, see pages 64 to 72.

Animals.—Healthy large sized rabbits.

Care of Animals.—*See page 255.*

Preparation of Experiment.—First completely anesthetize the animal with 0.75 grams per kilo of urethane, subcutaneously injected then connect the animal with apparatus as described on page 66.

Solutions.—All preparations to be tested should be made into solution suitable for intravenous injection.

The Active Principle (Tethelin) should be intravenously injected in doses of 0.05 gm. per kilo, dissolved in about 5 c.c. of normal saline solution.

Solutions anterior pituitary extract such as marketed for hypodermic injection may be injected without concentration or dilution. The usual dose is about 0.5 to 0.75 c.c. per kilo, injected intravenously.

Powdered Anterior Pituitary Desiccated.—A suitable solution of the extract of this substance should be made as follows: Place 20 grams of the powder in a small erlenmeyer flask and add 100 c.c. of 0.2 per cent. Acetic Acid. Shake occasionally during one hour. Slowly bring to boil and boil for about 15 seconds. Filter and allow to cool. Doses of 0.7 c.c. per kilo, of this solution should then be tested by intravenous injection.

Experiment.—After all preliminary arrangements have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood-pressure tracing is then started

on a slowly revolving drum. After obtaining a tracing of normal pressure about three inches in length, inject the solution to be tested into the femoral vein; take continuous tracing until the blood-pressure returns to normal. It will be noted from Fig. 59 that the intravenous injection of this product produces a fall in blood pressure and an increase in the amplitude of the tracing of the heart beat.

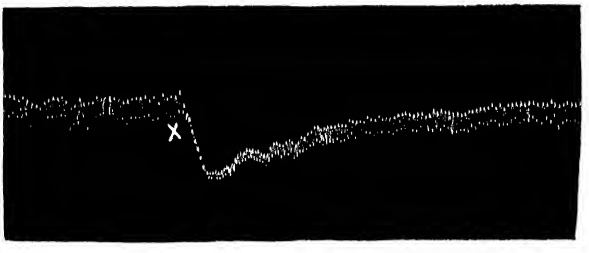

FIG. 59.—Action of Anterior Pituitary upon the blood pressure of a rabbit.

This method is *not satisfactory for the quantitative measurement* of the relative activity of two or more samples. It does, however, offer a satisfactory method of *qualitatively* demonstrating the presence of the typical action in the various preparations of the anterior lobe of the pituitary gland.

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CHAPTER VIII

CANNABIS

There are several commercial varieties of Cannabis sativa on the market, the most important of which are the Indian, African, Australian and American.

Formerly it was the general belief that only the Indian variety of Cannabis was of value therapeutically. It was also believed to be a distinct species, but it really differs so little from the other forms that botanists now consider them as merely varieties.

TABLE VII.—THE COMPARATIVE RESULTS OF BOTH CHEMIC AND PHYSIOLOGIC ASSAY OF THE PRINCIPAL THREE VARIETIES OF CANNABIS SATIVA

Cannabis indica		Cannabis africana		Cannabis americana	
Chemical assay, per cent.	Physiological assay, per cent.	Chemical assay, per cent.	Physiological assay, per cent.	Chemical assay, per cent.	Physiological assay, per cent.
12.2 resin...	.....	10.6 resin	100	6.4 resin	133
12.7 resin...	.....	16.77 resin	133.3	12.46 resin	100
14.25 resin..	100	14.2 resin	133.3	14.09 resin	100
12.81 resin..	100	8.6 resin	100	12.94 resin	133
13.04 resin..	80	10.0 resin	133	5.29 resin	100
13.4 resin...	66	17.5 resin	Less	6.55 resin	100
14.0 resin...	160	.....	than 20	10.79 resin	160
10.38 resin..	133	18.79 resin	100	.....	250
15.07 resin..	133	17.78 resin	83	.....	114
.....	200	10.71 resin	133	17.06 resin	114
.....	140	.....	.....	10.87 resin	260
13.02 resin..	133	.....	.....	8.53 resin	114
.....	200				
.....	266				
.....	200				
9.87 resin..	125				

There exists a difference of opinion as to the comparative physiologic activity of the different varieties. So far as assays on dogs are concerned, there seems to be very little choice between them (see Table VII).

Table VII shows that although there seems to be little choice between the different varieties of Cannabis, there *is* a marked variation in the activity of different samples of the *same* variety. It also shows a large variation between the chemic and physiologic results, thus proving the fallacy of the formerly common practice of standardizing preparations of Cannabis according to their resin content. It can readily be seen, therefore, that the only reliable index to the therapeutic value of this drug is the *physiologic* assay.

H. C. Wood[1] states, "Extract of hemp is a very unsatisfactory drug from the fact that 1/8 of a grain of one extract will produce decided intoxication, and many grains can be taken of another extract that cannot be distinguished physically or chemically from the first specimen." This objection, however, can be entirely eliminated by physiologic standardization as by this means every preparation can be brought to the same strength *before* its use on the human.

Standardization of Cannabis

This assay depends upon the characteristic action of the drug upon the central nervous system, in which changes are induced causing a peculiar train of symptoms. These are divided into three typical stages: (1) one of excitability; (2) one of inco-ördination; (3) one of lassitude and sleep.

Shortly after receiving a dose of suitable size of cannabis indica the animal generally vomits and then becomes excitable. In from one to two hours inco-ördination follows; the dog loses control of its legs and of the muscles supporting the head, so that, when standing, the feet are usually spread apart to maintain balance. (See Fig. 61.) When nothing occurs to attract attention the head droops and body sways from side to side or anteroposteriorly. A distinct ataxia is present when walking, and, when severely affected, the animal will stagger and fall. If, however, it is spoken to sharply

[1] Wood: Therapeutics, Its Principles and Practice, p. 117, 14th edition.

or attention is drawn to anything of interest it may momentarily recover and the typical effects of the drug disappear, but in a few minutes again relapses into the former condition. As the action

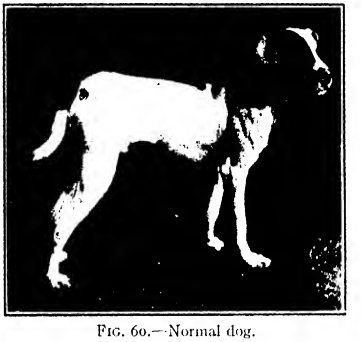

FIG. 60.—Normal dog.

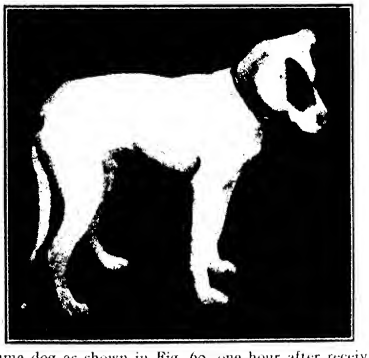

FIG. 61.—Same dog as shown in Fig. 60, one hour after receiving a dose of active cannabis. This figure clearly illustrates the stage of inco-ördination produced by cannabis. That the dog has lost control of the hind legs and of the muscles supporting the head can be noted by the dropping of the head and hind quarters. Also note that the legs are spread apart in order to maintain balance.

of the drug progresses the muscular inco-ördination becomes greater and the animal passes into the third stage; the depression and lassitude are increased until finally the animal sinks to the floor as if

exhausted and passes into deep, undisturbed sleep. After several hours the effects of the drug slowly disappear and the normal condition returns.

For standardization purposes the end-reaction to be observed is one just sufficient to produce muscular inco-ördination in a dog.

Animals.—Short-haired dogs of medium size (6 to 15 kilos) are well adapted for this work. The U.S.P. requires that the dogs weigh less than 15 kilograms. As a rule fox terriers serve very well

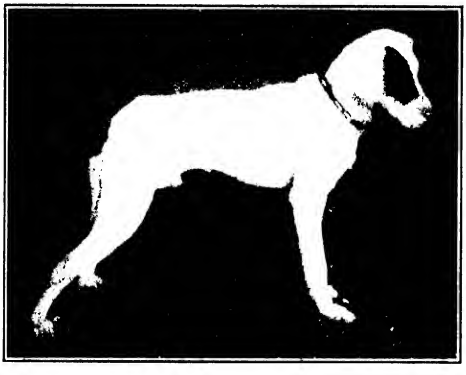

FIG. 62.—Same dog as shown in Figs. 60 and 61, one and a half hours after receiving a dose of active cannabis indica. This figure shows the animal when severely affected, and about to fall forward.

and readily show the different stages of the drug's action because of their comparative high cerebral development but any dog may prove satisfactory.

Animals for assay purposes should be selected with great care, it being necessary to pick out those that are healthy, intelligent, quiet, and which have shown by previous tests that they are easily susceptible to the action of the drug. The dogs should be at least one year old and must be kept under the best sanitary conditions.

Each series of tests should be conducted by the same operator. Before using the dogs for actual work, he should study each animal in order to familiarize himself with the behavior, peculiarities, etc.,

of each dog under normal conditions in order that he may recognize more certainly deviations from the normal. The same animal may be used many times, provided that thirty-six hours are allowed to elapse between doses in order that the animal may *completely* recover from the effects of the previous dose.

Although the animals never appear to lose their susceptibility, it is not advisable to use a dog for more than six months, and care should be taken to allow one week to elapse between assays.

Care of Animals.– See page 240.

Preparation of Experiment.–Select and weigh several animals of known susceptibility to the "standard fluidextract," and withhold all food for at least twelve hours previous to the time of administration of the drug. Water should be allowed.

Dogs differ considerably in susceptibility to the drug and therefore, immediately after receiving a lot of dogs for testing purposes their individual susceptibility to the "Standard Fluidextract Cannabis" should be determined. Each dog should be numbered with metal tags attached to the collar and then receive, at intervals of three to four days, doses not exceeding 0.1 c.c. per kilo body weight of animal, of the *standard* until the smallest amount per kilogram body-weight of animal is determined that will produce an action just sufficiently pronounced to bring on the stage of inco-ördination. This is distinguished by a slight ataxia when walking and a drooping of the head and gentle swaying of the body while at rest. All animals which are not of standard susceptibility may be discarded or the smallest amount of the standard required to produce inco-ördination in each animal may be recorded and used for comparison with the amounts of the unknown required to produce the same effects upon the same dog. The susceptibility of each dog to the "standard" should be checked at least every two months.

Preparation of Drug for Administration.– All preparations to be tested should be carefully diluted or concentrated until 1 c.c. of the preparation represents one gram of drug.

The doses to be given are accurately measured directly into hard gelatin capsules. When a crude drug is to be tested a representative sample should be finely ground and then made into a fluid extract by the U.S.P. method.

Method of Administering.—The drug is administered internally by means of a small capsule. The animal's mouth is opened by forcing the thumb and index finger of the left hand between the jaws, back of the teeth. The capsule is then placed on the back of the tongue

FIG. 63.—Method of administering capsules to dogs.

with the right hand and the mouth quickly closed; while still holding the mouth shut, the animal can be made to swallow the capsule immediately by slapping it on the throat.

Actual Standardization.—Administer to a series of three dogs, of known susceptibility to the standard, doses of the unknown prepara-

tion of 0.025, 0.03 and 0.035 c.c. respectively per kilogram body weight of animal. The animals are then placed in a room where they will be undisturbed, remote from noise and excitement and separated so that they cannot see each other. (See Extra Large Size Animal Cage Fig. 91 p. 237.)

At the end of one hour careful observations should be made. It is important that the observations be made between one and one and one half hours after the administration of the drug as the maximum inco-ördination is reached in about this time.

While making observations the operator must remain motionless. This is very important because in most cases (unless the dose be excessive) the slightest movements of the operator, noise outside of the room, movement or barking of other animals, will attract the dogs attention producing a momentary recovery, and render it impossible to note the typical action of the drug until the animal again relapses into its former condition.

The operator, therefore, must not expect to note the typical effects of the drug immediately upon entering the room. He should enter the room, take a seat from which the animal may be observed, and then remain motionless until the animals no longer are attracted by his presence. The results of the observations should then be recorded.

The results of this preliminary test enables the operator to form some idea as to the strength of the preparation. Basing the dosage upon these results, other series of dogs are given progressively increasing or decreasing doses, as the case may be, until the smallest amount is found which will produce approximately the same degree of incoördination as was produced in the same dog when the "standard" was administered. The probable "minimum effective dose" of the preparation unless it deviates considerably from that of the standard, is generally obtained by one or two series of doses. In order to determine whether or not this is the true "minimum effective dose" this result is checked by carefully administering doses to a series of two more dogs; one with the smallest dose that produced the standard effect, and one with the largest dose that did not produce apparent effects. If, however, the results of this last series do not check the previous results, further correction must be made.

After thus determining the "minimum effective dose" of the unknown preparation its relative strength is calculated by comparing it with the "minimum effective dose" of the standard preparation, as previously determined for each individual dog.

Official Standards.—The following are the official standards for cannabis and its official preparations:

Cannabis U.S.P., in the form of the fluidextract, administered by the mouth to dogs in doses not exceeding 0.1 c.c. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by the same dose of the standard fluidextract of cannabis, prepared as directed below.

Standard Fluidextract of Cannabis.—The U.S.P. directs that the standard fluidextract, as supplied by the Bureau of Chemistry, U. S. Department of Agriculture, be prepared as follows:

"Prepare a composite fluidextract, representing at least ten different lots of Cannabis, conforming to the official botanical description, and administer this fluidextract in gelatin capsules to dogs by the mouth. This standard fluidextract must be so adjusted that it will produce incoördination in dogs which have been found to be susceptible to the action of Cannabis when administered in doses of 0.03 c.c. for each kilogram of body weight of dog."

Extract of Cannabis U.S.P., administered by the mouth to dogs, in doses not exceeding 0.004 gm. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by 0.03 c.c., for each kilogram of body weight of dog, of the standard fluidextract of cannabis prepared as directed under *Cannabis.*

Fluidextract of Cannabis U.S.P., administered by the mouth to dogs in doses not exceeding 0.1 c.c. for each kilogram of body weight of dog, produces the same degree of incoördination as that produced by an equivalent dose of the standard fluidextract of cannabis, prepared as directed under *Cannabis.*

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CHAPTER IX

PISCIDIA ERYTHRINA (JAMAICA DOGWOOD)

Although the amount of Jamaica dogwood prescribed and used by the present-day practitioner is very small as compared with such drugs as cannabis and opium, which possess a somewhat similar but more powerful action, the drug is still used in appreciable quantities.

Any drug, however, which is worthy of being used as a medicinal agent should be standardized either by chemical or biological methods.

As the literature contained no satisfactory method of assaying Jamaica dogwood the author and one of his co-workers conducted a series of experiments with the object of developing, if possible, either a chemical or a biological method for standardizing this drug.[1]

Since the principal end to be accomplished by the assay of a drug or its preparations is to secure a *means of measuring its therapeutic efficiency*, a chemical method fails of its purpose unless some direct and constant ratio exists between the figures obtained by the assay process and the therapeutic activity of the drug. For this reason it was necessary for us to first develop a satisfactory biologic method for measuring the therapeutic *activity* of the drug. Without a satisfactory biologic method it is impossible to determine whether or not the substance isolated by chemical means bears any relation to the activity of the drug.

We, therefore, first devoted our attention to the physiologic action.

Physiologic Action

The researches of Ott[2] and Nagle[3] show that Jamaica dogwood possesses a marked sedative, analgesic and hypnotic action.

[1] "The Standardization of Piscidia Erythrina (Jamaica Dogwood)," by Paul S. Pittenger and George E. E'we, The American Journal of Pharmacy, September, 1919.

[2] Ott, Isaac: *Therapeutic Gazette*, 1883, supplement to March number, pages 12 to 17 inc.

[3] Nagle, A. C.: *Druggists Circular*, Feb., 1881, p. 18.

Of the three actions mentioned, the hypnotic effect presented itself as the most likely means of physiologic standardization.

The similarity between the actions of Jamaica dogwood and cannabis suggested the possibility of employing similar methods of standardization.

A fluid extract of the drug was accordingly administered in capsules to dogs and found to produce incoördination and ataxia similar to that produced by cannabis.

The hypnotic effect of Jamaica dogwood, however, was found to be less than that of cannabis, as it required approximately 17 times as much Jamaica dogwood to produce the same degree of incoördination in dogs as that produced by cannabis.

For standardization purposes the end reaction to be observed is one just sufficient to produce muscular incoördination in a dog.

The details of the method employed follow:

Animals.—Short-haired dogs of medium size (6 to 12 Kilos) are well adapted for this work. They show the different stages of the drug's action because of their comparative high cerebral development.

Animals for assay purposes should be selected with great care, it being necessary to pick out those that are healthy, intelligent, quiet, and which have shown by previous tests that they are easily susceptible to the action of the drug.

After several dogs have been selected, the operator, before using them for actual work, should study each animal in order to familiarize himself with the behavior, peculiarities, etc., of the dog under normal conditions. The same animal may be used many times, provided that twenty-four to thirty-six hours are allowed to elapse between doses in order that the animal may *completely* recover from the effects of the previous dose.

Although the animals never appear to lose their susceptibility, it is not advisable to use a dog for more than six months, and care should be taken to allow one week to elapse between assays.

Preparation of Experiment.—Select and weigh several animals which have been found easily susceptible to the action of Jamaica dogwood, and withhold all food for at least twelve hours previous

to the time of administration of the drug. Water should be allowed.

Preparation of Drug for Administration.—Tinctures, solid, powdered, and fluid extracts, are weighed or measured directly into hard gelatin capsules. When a crude drug is to be tested a representative sample should be finely ground and then made into a fluidextract.

Method of Administering.—The drug is administered internally by means of a small capsule. The animal's mouth is opened by forcing the thumb and index finger of the left hand between the jaws, back of the teeth. The capsule is then placed on the back of the tongue with the right hand and the mouth quickly closed; while still holding the mouth shut, the animal can be made to swallow the capsule immediately by slapping it on the throat.

Actual Standardization.—Administer to a series of three selected dogs 9/10, 10/10 and 11/10 of the standard dose of the preparation to be tested, for each kilo body weight of animal. The animals are then placed in a room where they will be undisturbed and are remote from noise and excitement; careful observation should be made and the results recorded during four or five hours.

If this preliminary test shows that the drug is either above or below standard strength other dogs are given progressively increasing or decreasing doses, as the case may be, until the smallest dose per kilo body weight is found which will produce an action just sufficiently pronounced to bring on the stage of incoördination. This is distinguished by a slight ataxia when walking and a drooping of the head and gentle swaying of the body while at rest. The relative strength of the preparation tested is then computed between the "minimum dose" and the "standard minimum dose" by simple proportion.

The personal equation plays an important part in this assay just as in cannabis, since the accuracy of the test depends largely upon the experience of the operator and his ability in determining just when the effects of the drug manifest themselves. In the hands of an experienced operator, therefore, results may be obtained which will show, with fair accuracy, the relative value of any preparation of Jamaica dogwood.

Tentative Standard

In order to determine the average amount of the drug per kilo required to produce incoördination in dogs and to set a tentative standard for assay purposes, ten different samples of fluid extract obtained from the various pharmaceutical manufacturing houses in the United States were assayed by the above method and were found to produce incoördination in dogs in the following doses:

Sample No.	
1	0.7 c.c. per k. body weight of dog
2	0.6 c.c. per k. body weight of dog
3	0.5 c.c. per k. body weight of dog
4	0.4 c.c. per k. body weight of dog
5	0.7 c.c. per k. body weight of dog
6	0.5 c.c. per k. body weight of dog
7	0.4 c.c. per k. body weight of dog
8	0.4 c.c. per k. body weight of dog
9	0.5 c.c. per k. body weight of dog
10	0.5 c.c. per k. body weight of dog

You will note, therefore, that the average of the above ten samples is 0.52 c.c. per k. We have, therefore, adopted the following tentative standard:

"Fluid extract of Jamaica dogwood should be of such strength that it will produce incoördination in dogs in doses of 0.55 c.c. per kilo body weight of animal and should not produce incoördination in doses less than 0.5 c.c. per kilo, the drug being administered by capsule after fasting the animal for 12 hours.

The above experiment also shows the wide variation in the strengths of the commercial preparations on the market and proves the necessity for standardizing preparations of this drug.

Chemical Investigation

The only physiologically active constituent which could be found credited to Jamaica dogwood in this investigation was the crystalline substance "piscidin." Piscidin is credited by Berberich[1] as having

[1] Berberich, Herman: American Journal of Pharmacy, Sept., 1898, pp. 425–427.

the formula $C_{29}H_{24}O_8$. Berberich also states that Edward Hart[1] by treating the fluid extract of the bark of Jamaica dogwood with slaked lime, obtained a crystalline substance, which he considered to be the active principle of the bark.

We repeated the work of Hart and Berberich with the exception of the melting point determination and elementary analysis and obtained the same results noted by them.

A modification of the lime method of isolation of piscidin was developed in the hope that it might be applicable to the assay of fluid extract of Jamaica dogwood by chemical means. The details of this method are given in the paper referred to.

In order to ascertain the relation of the piscidin recovered by this process to the activity of the fluid extract from which it was obtained, the amount of piscidin yielded by 100 c.c. of a fluid extract was redissolved in hot alcohol, then diluted to 100 c.c. with the weakest strength alcohol which would keep the piscidin in solution and this solution was then tested physiologically in comparison with the original fluid extract. In two experiments the piscidin recovered by assay represented 55 per cent. and 62.5 per cent. respectively of the activity of the fluid extracts.

Ten samples of fluid extracts representing all of the larger pharmaceutical manufacturing houses in the United States were assayed by this lime process in comparison with the physiological assay process in order to determine whether or not the piscidin content paralleled the physiologic activity. The results of these assays follow:

Sample No.	Chemical Assay.	
1.....	0.219 per cent. impure piscidin	0.7 c.c. per kilo.
2.....	0.235 per cent. impure piscidin	0.6 c.c. per kilo.
3.....	0.450 per cent. impure piscidin	0.5 c.c. per kilo.
4.....	0.460 per cent. impure piscidin	0.4 c.c. per kilo.
5.....	0.507 per cent. impure piscidin	0.7 c.c. per kilo.
6.....	0.620 per cent. impure piscidin	0.5 c.c. per kilo.
7.....	0.620 per cent. impure piscidin	0.4 c.c. per kilo.
8.....	0.650 per cent. impure piscidin	0.4 c.c. per kilo.
9.....	0.670 per cent. impure piscidin	0.5 c.c. per kilo.
10.....	0.680 per cent. impure piscidin	0.5 c.c. per kilo.

[1] Hart, Edward *American Chem. Journ.*, 1883, p. 39, also *Therapeutic Gazette*, 1883, pp. 97, 98.

These comparisons show that the piscidin content is not in direct ratio to the physiologic activity and therefore make evident the impossibility of using the lime method of isolating piscidin, as a means of chemically standardizing Jamaica dogwood preparations.

Conclusions

The result of these experiments would tend to prove, therefore, that we are without a reliable chemical means of accurately standardizing Jamaica dogwood preparations but that they can be accurately standardized by physiological means as outlined.

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CHAPTER X

EPHEDRA AND EPHEDRINE

Ephedrine is an active alkaloid originally isolated in an impure form from the Asiatic drug, *Ma Huang* by Yamanashi. It was first isolated in the pure form by Nagai.[1]

Ma Huang, identified as Ephedra vulgaris var. helvetica has been used in the practice of medicine in China for more than five thousand years but remained practically unknown until revived by the vast researches and publications of pharmacological and clinical studies by Chen and Schmidt and Chen[2] during 1924 to 1926.

The emperical formula for ephedrine is $C_{10}H_{15}ON$; its chemical structure most probably is 1-phenyl-2-methylamino-propanol-1 $C_6H_5.CHOH.CH(NHCH_3)CH_3$.

It will be noted by the chemical composition of ephedrine that it is allied closely to epinephrine. (See p. 61.) In many ways it also simulates epinephrine in its physiologic action. Its effects on the circulation, smooth muscle and secretions are due to sympathetic stimulation and resemble *qualitatively* those of epinephrine. In addition it stimulates the central nervous system and depresses the heart, but these effects are elicited ordinarily only by toxic doses. It produces a **rise in blood pressure** due to **vasoconstriction and cardiac stimulation.** It **stimulates uterine muscle** and relaxes the bronchial muscle. It also possesses **mydriatic action.**

Quantitatively, however, there are important differences in the effects of epinephrine and ephedrine. Ephedrine is much less

[1] Nagai, Pharm. Ztg., 32, 700, 1887.

[2] Chen and Schmidt, Proc. Soc. Exptl. Biol. Med. 21, 351, 1924; J. Pharmacol, 24, 339, 1924; China Med. J. 39, 382, 1925; Chen, Proc. Soc. Exptl. Biol. Med. 22, 203, 1924; 22, 404, 1925; 22, 568, 1925; 22, 570, 1925; Jour. A. Ph. A., 14, 189, 1925; J. Pharmacol. 26, 83, 1925; 27, 61, 1926; 28, 77, 1926; 27, 87, 1926; 27, 239, 1926.

powerful than epinephrine but its effects are much more presistent. (See Figs. 64 and 65.) These figures show that an intravenous injection of 0.2 c.c. of a 1–10,000 solution of epinephrine produced a greater rise in blood pressure than the same size dose of a 3 per cent. solution of ephedrine. On the other hand the blood pressure returned to normal within five minutes after the injection of the epinephrine whereas the pressure was considerably above normal one hundred and twenty five minutes after the injection of the ephedrine.

Ephedrine also differs from epinephrine in that *it is active when administered orally.* Epinephrine given by stomach does not produce appreciable systemic reactions in normal animals even when administered in large doses. According to Rowe[1] "This is due partially to the rapid destruction of the adrenalin in the stomach before it can be absorbed and to the prevention of absorption by the powerful vasoconstrictor action on the vessels of the alimentary tract."

The effect of oral administration upon the blood pressure is shown in Fig. 66.

Ephedrine further differs from epinephrine in that its solutions are stable indefinitely even when exposed to light and air and are not decomposed on boiling. Solutions of epinephrine decompose rapidly upon exposure to light, air and on boiling. For this reason commercial solutions are protected by amber bottles and are sealed in an atmosphere of carbon dioxide.

Ephedrine combines readily with acids to form the sulphate, chloride, nitrate, acetate, etc. There is no important therapeutic differences between the various salts but in view of the fact that most alkaloids may be purified to a higher degree in the form of their sulphates, most of the manufacturers are marketing ephedrine sulphate in preference to the hydrochloride or other salts.

According to Rowe the M.L.D. of ephedrine sulphate is about 400 mg. per kg. body weight of white mice.

Ephedrine, therefore, although somewhat similar to epinephrine chemically and in its qualitative actions is quite different in its quantitative action.

[1] Rowe: "The Comparative Pharmacologic Action of Ephedrine and Adrenalin," Jour. A. Ph. A., 10, 912, 1927.

Present indications are that its therapeutic usefulness will be more limited and in many cases quite distinctive from those of Epinephrine.

Experiment. (*a*) **The effect upon the circulation from** *intravenous injection* may be demonstrated by the following experiment.

Apparatus Necessary for Experiment, Animals, Preparation of Experiment and Method of Injection. Same as required for the standardization of epinephrine (see page 64).

Preparation of Solution.— Make a 3 per cent. solution of ephedrine sulphate in distilled water.

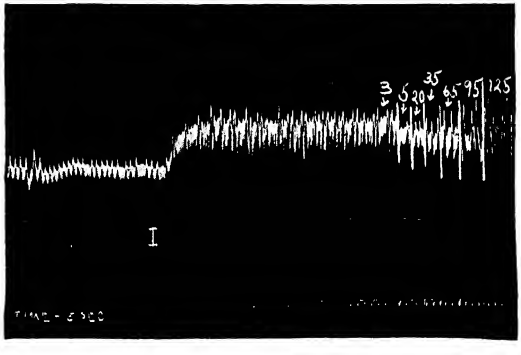

FIG. 64.—Effect of intravenous administration of ephedrine upon the Circulation. 0.2 c.c. of a 3 per cent. solution of ephedrine sulphate intravenously injected at I. Figures above tracing indicate the number of minutes after injection.

Method.—After all preliminary arrangements have been made bring the writing point of the manometer to bear upon the smoked paper of the kymograph. The blood pressure tracing of normal pressure is then started on a slowly revolving drum. After obtaining a tracing of normal pressure about 3 in. in length, inject 0.2 c.c. of the above solution into the femoral vein; take continuous tracing for three to five minutes and then stop the drum; allow the drum to revolve for about 1 in. every ten or fifteen minutes for two hours (see Fig. 64). It will be noted that immediately after the intravenous injection of ephedrine the blood pressure rises sharply due to

the constriction on the vessels. It will be noted that after the pressure reaches its maximum it is sustained for a prolonged period and does not quickly return to normal like epinephrine (see Fig. 65).

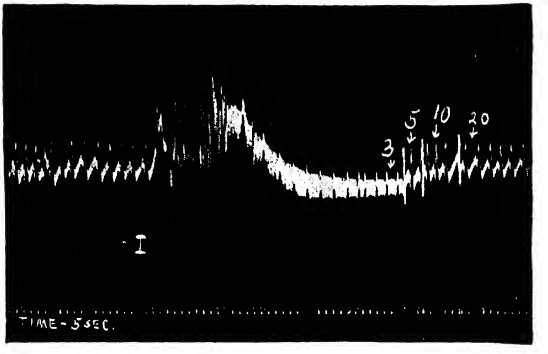

FIG. 65.—Effect of intravenous administration of epinephrine upon the circulation. 0.2 c.c. of a 1–10,000 solution of epinephrine hydrochloride injected intravenously at I. Figures above tracing indicate the number of minutes after injection.

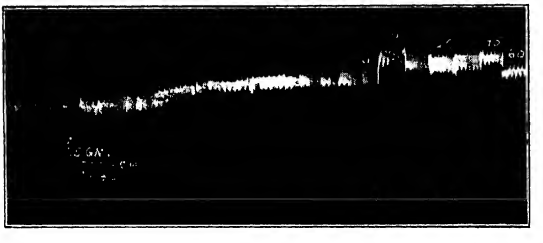

FIG. 66.—Effect of oral administration of ephedrine upon the circulation. 0.75 gm. ephedrine sulphate administered orally at X. Figures above tracing indicate the number of minutes after injection.

Compare this action with *the action of epinephrine upon the circulation* by repeating the experiment upon another dog, injecting 0.2 c.c. of a 1–10,000 solution of epinephrine in place of the ephedrine. Record in the same manner. (See Fig. 65.)

Experiment. (*b*) **The effect upon the circulation,** *from oral administration* may be demonstrated as follows:

Starve a medium sized dog for a least twenty four hours; arrange apparatus, anesthetize animal and prepare for making blood-pressure tracing in the same manner as described above. Record normal blood pressure and then administer a dose of 0.75 gm. of ephedrine sulphate in 25 c.c. of water, by stomach tube. Record blood pressure every 20 minutes.

Experiment. (*c*) **Stimulation of Cardiac Muscle.** –The effect of ephedrine upon the cardiac muscle may be demonstrated by perfusing the excised mammalian heart with blood containing ephedrine. For apparatus necessary, animals, preparation of experiment and technique employed, see description under "Isolated Mammalian Heart," page 317. Proceed as under Epinephrine page 62, using a 0.05 per cent. solution of ephedrine sulphate in Locke-blood solution.

Experiment. (*d*) **Mydriatic Action.**– The mydriatic action of ephedrine was first obtained by Miura and may be demonstrated by tests on the rabbit's or cat's eye.

For apparatus necessary, animals, preparation of experiment and technique employed see "Munch Method for Biologic Assay of Mydriatics" page 158. Use a 1 per cent. to 5 per cent. solution of Ephedrine Sulphate.

Standardization of Ephedra and Ephedrine

The purity of the various salts of ephedrine may be established by chemical methods. It is, however, advisable to verify these results by biologic tests, due to the fact that when the compound is synthesized the racemic variety is obtained which is not so active as the levo-variety which occurs in the natural product.

A marked variation in the activity is also found in the crude drug. There are many varieties of Ephedra[1] some of which are practically inactive. The analysis of a number of samples of Ephedra vulgaris were found to have an alkaloidal content ranging from 0.2 per cent.

[1] "The Occurrence and Alkaloidal Content of Various Ephedra Species" by Nielson, McCousland and Spruth, Jour. A. Ph. A., 16, 1927, 288.

to 0.9 per cent. of total alkaloid. (Nielson *et al.*) Later investigations by Feng and Read[1] show that when fully extracted the total yield of alkaloids may exceed 1 per cent.

Of the various physiologic actions above mentioned there are two which present themselves as possible means of biologic standardization, *i.e.*, the mydriatic action and the action on the blood pressure. The action chosen for testing the drug should be governed largely by the therapeutic effects it is desired to produce.

For example, if it is to be used as suggested by Middleton and Chen[2] in connection with other drugs for the purpose of producing mydriasis, it should be standardized according to its mydriatic action. If it is to be used for its effects on circulation, smooth muscle and secretions due to its sympathetic stimulation it should be tested for its effect upon the blood pressure.

STANDARDIZATION OF MYDRIATIC ACTION OF EPHEDRA OR EPHEDRINE

For standardizing ephedrine according to its mydriatic action proceed according to the method described under Chapter XI "Myotics and Mydriatics" page 157. If the crude drug is to be assayed it must first be made into a fluidextract.

BLOOD-PRESSURE METHOD FOR STANDARDIZING EPHEDRA OR EPHEDRINE

It is difficult to devise a quantitative blood-pressure method owing to the peculiarity of the drug in that the size of the dose does not accurately govern the resultant rise in blood pressure, also to the fact that in many cases a tolerance is exhibited after the first injection of the drug. This variation in response and the tolerance exhibited after the administration of comparatively large doses was found by Feng and Read[1] to be practically eliminated if the drug is adminis-

[1] "The Ephedrine Assay of Chinese Ephedra" by C. T. Feng and B. E. Read, Jour. A. Ph. A., 16, 1927, 1030.

[2] Middleton and Chen, Arch. Int. Med. 39, 1927, 385.

tered in small doses. They found that small doses will produce an appreciable rise in blood pressure. Also that small doses if repeated at sufficiently long intervals show a like effect accurate enough for assay purposes. This drug may, therefore, be assayed as follows:

Apparatus Necessary for Experiment; Animals; Preparation of Experiment and Method of Injecting.—Same as required for the standardization of epinephrine. (See page 64.)

Preparation of Standard Solution.—Prepare a standard solution of pure ephedrine sulphate or hydrochloride obtained from authentic samples of Ephedra vulgaris, var. helvetica, containing 0.001 gm. per c.c.

Preparation of Unknown Solution.—The solutions obtained from the chemical assay of the drug should be diluted so that 1 c.c. contains 0.001 gm. of the theoretical alkaloids present.

If it is a commercial sample of one of the salts of ephedrine it should be made into a solution containing 0.001 gm. per c.c.

If a crude drug is to be tested, without chemically extracting the alkaloids in the pure form, a sample of the drug should be made into a fluidextract.

Actual Standardization.—The blood-pressure tracing is started on a slowly revolving drum. After obtaining a tracing of normal pressure about 3 in. in length, inject 1 c.c. of the above standard solution and take continuous tracing for 10 minutes and then stop the drum. After 1 hour inject 1 c.c. of the solution obtained from the chemical assay of the unknown sample, or 0.1 c.c. of the fluidextract. Take continuous tracing for 10 minutes and again stop drum. The rise in pressure produced by the unknown solution is compared with that produced by the standard solution. The size of the injection of the unknown solution is then increased or decreased, allowing one hour between injections, until that dose of the unknown is found which will cause the same rise in pressure as that produced by the standard solution. The relative strengths of the two preparations are in inverse proportion to the doses required to produce similar rises.

Use of Several Animals.—In order to rule out any inaccuracy due to the possibility of a tolerance being exhibited after the first dose, the assay should be repeated on a second dog. In the second experiment the dose of the unknown, which was found effective in the first

experiment, should be administered *first* and the standard solution *second*. If the results of the two experiments do not check the experiment should be repeated upon additional animals.

Standard.—The solution of the unknown ephedrine should produce the same rise in blood-pressure as that produced by equal doses of the standard solution.

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CHAPTER XI

MYOTICS AND MYDRIATICS

The dilation of the pupil is known as *mydriasis* and the constriction of the pupil as *miosis*. Drugs which cause a dilation of the pupil are termed *mydriatics* and those which cause a constriction are termed *myotics*.

According to Sollmann[1] the causes of Mydriasis and Miosis and the mechanisms through which the pupils may be affected are as follows:

Causes of Mydriasis.—Mydriasis can be due to any of the following causes: Stimulation of the sympathetic mechanism (cocain). Paralysis of the oculomotor mechanism, peripheral (atropin) or central (asphyxia). Increased inhibition of the oculomotor center (excitement).

Causes of Miosis.—These may consist in: Stimulation of the oculomotor mechanism, peripheral (pilocarpin, physostigmin), or central (picrotoxin). Lessened inhibition of the oculomotor center (sleep, narcosis, morphin). Paralysis of the sympathetic mechanism (practically unimportant).

Mechanisms through which the pupils may be affected are as follows:

Dilator Mechanism	Constrictor Mechanism
Sympathetic center	Osculomotor center
Sympathetic and long ciliary nerve	Osculomotor and short ciliary nerves
Superior cervical ganglion	Ciliary ganglion
Postganglionic fibres	Postganglionic fibres
Endings in radial muscle	Endings in sphincter muscle
Fibres of radial muscle	Fibres of sphincter muscle

The chemical methods for the quantitative valuation of myotics and mydriatics are of doubtful accuracy. This is especially true in the case of tablets where a very minute quantity of the myotic

[1] Sollmann: Manual of Pharmacology.

or mydriatic is present in combination with other alkaloids or medicinal agents. In most cases the other alkaloids in the tablets interfere with the chemical assay of the myotic or mydriatic. These substances, however in most cases will not produce miosis or mydriosis and, therefore, will not interfere with a biologic assay based upon the effects of a solution of the tablets, upon the eye of a cat.

Although cats, rabbits, frogs, etc., have been used for years to demonstrate the qualitative effects of this class of drugs upon the pupil of the eye, the first definite application of this action to quantitative biologic assays was made by J. C. Munch.[1]

Munch Method for Biologic Assay of Myotics and Mydriatics.—This method consists in determining the smallest amount of a solution of the unknown required to produce the same degree of miosis or mydriasis as that produced by a standard solution of known strength.

Apparatus Necessary for Experiment.—One c.c. Mohr pipettes, graduated in 0.01 c.c., with slender tips which deliver exactly 0.05 c.c. per drop, 100-watt nitrogen-filled electric lamps or equally intense illumination and volumetric flasks.

Animals.—Adult cats in good physical condition, weighing over 1500 grams, and accustomed to being handled.

Preparation of Sample.—Dissolve a representative number of tablets, or a sufficient quantity of powder, in approximately neutral distilled water, to make a solution containing one milligram of the alkaloid per c.c. of solution. In case the alkaloids themselves are taken, the equivalent quantities of acid are added to convert them into the corresponding acid salts. Add two drops of approximately N/50 acid per 50 c.c. of solution.

For great accuracy, chemical assay results upon the sample should be followed in the preparation of solutions; where such accuracy is unnecessary, the declaration of strength on the label may be accepted as the basis for the preparation of the solution.

Determination of Cat's Threshold.—Place a cat about one foot from a 100-watt electric lamp, and determine the maximum contractility of its pupils under this condition. Drop 0.05 c.c. of the freshly

[1] Munch: Jour. A. O. A. C., Vol. X, pages 383–386, 1927.

prepared *standard* mydriatic solution, obtained by diluting the 1 mg.-per-c.c. *solution*, into the outer margin of one eye, leaving the other eye untreated as a control. Compress the inner canthus, while opening and closing the lids, until the fluid has just apparently disappeared (10 to 30 seconds). Return cat to cage.

One and two hours after application(for atropine, 3 and 4 hours also), place cat under the same conditions, and note any differences in diameter between the pupils of the treated and the untreated eyes. A satisfactory reaction is produced when the pupil of the treated eye is just perceptibly wider (0.5 to 1.0 mm.) than the pupil of the untreated eye. The same eye should not be used for another assay for at least 24 hours.

The following concentrations were found by J. C. Munch to give threshold reactions.

TABLE VIII.—MINIMUM EFFECTIVE CONCENTRATIONS, MG. PER LITRE. ALL RESULTS CALCULATED IN TERMS OF THE ALKALOIDS

\+ Mydriatics.
° Miotics

+Alypine	No effect	+Digitalin (German)	No effect
+Anesthesine	No effect	+Digitonin	No effect
+Apomorphine HCl	No effect	+Digitoxin	No effect
+Apothesine	No effect	+Duboisine sulfate	1.6
°Arecoline HBr	(10,000)		
+Atropine sulfate	12	+Emetine	No effect
		+Ephedrine sulfate	(50,000)
+Barbital	No effect	+Pseudo-ephedrine sulfate	(80,000)
+Bebeerine	No effect	°Eserine	(10)
+Berberine	No effect	+Eucatropine	No effect
+Brucine sulfate	No effect	+Euthalmin HCl	50,000
+Butesin	No effect	+Ergotoxine phosphate	No effect
+Butyn	No effect		
		+Gelseminc resin	No effect
+Cephaeline HCl	No effect	+Gelseminine HCl	No effect
+Chloral hydrate	No effect		
+Cinchonidine sulfate	No effect	+Heroine	No effect
+Cinchonine sulfate	No effect	+Homatropine HBr	200
+Cinchotoxine HI	No effect	+Hydrastine	No effect
+Cocaine HCl	60	+Hyoscine	0.4
+Colchinine	No effect	+Hyoscyamine	4.0
+Cotarnine HCl	No effect		
+Curare	No effect	+Methyl atropine bromide	400
+Chloratone	No effect		
		+Narceine HCl	No effect
+Daturine sulfate	12	+Narcotine HCl	No effect
+Dionine HCl	No effect	+Nicotine $ZnCl_2$	No effect

TABLE VIII.—*Continued*

+ Mydriatics.
° Miotics.

+Novocaine	No effect	+Solanin	No effect
		+Sparteine syrup	No effect
+Papaverine	No effect	+Stovaine	No effect
°Physostigmine	(10)	+Strychnine sulfate	No effect
°Pilocarpine	(25,000)	+Sulfon-methane (sulfonal)	No effect
+Piperine	No effect		
+Psicaine	No effect	+Theobromine	No effect
		+Theophylline-sodium	No effect
+Quercitrin	No effect	+Tropocaine	No effect
+Quinidine sulfate	No effect	+Tutocaine	No effect
+Qinotoxine HCl	No effect		
+Quinine sulfate	No effect	+Urethane	No effect
+Salicaine	No effect	+Veratrine	No effect
+Santonine	No effect		
+Saponin	No effect	+Yohimbine HCl	No effect
+Sapotoxine	No effect		

All of the alkaloids listed in the above table were tested by Dr. Munch in order to determine whether they acted as mydriatics, miotics or had no effect at all upon the cats eye. This information is invaluable in the assay of tablet combinations as it supplies information as to the alkaloids which do not effect the pupil of the cat's eye.

If the concentrations shown in Table VIII fail to produce a satisfactory reaction, the test is repeated with a stronger or weaker solution, until the Minimum Effective Concentration is found. The Minimum Effective Concentration may vary somewhat for different cats, but is essentially constant for the same cat.

Actual Standardization.—Dilute the 1 mg.-per-c.c. solution to be tested to the Minimum Effective Concentration for the cats to be tested, and drop 0.05 c.c. of this dilution into one eye of the cat, following the same procedure as in the determination of the Minimum Effective Concentration. Also prepare stronger and weaker solutions, and apply to one eye of other cats. Test various concentrations, until one is obtained which just produces satisfactory mydriasis of the same degree as the standard solution, when tested on two or more cats.

Multiply the milligrams per c.c. found to be the cat's Minimum Effective Concentration by the dilution employed, to obtain the milligrams of alkaloid present in each c.c. of the original solution. Knowing that the original solution was made to contain 1 milligram of alkaloid per c.c., calculate the quantity of mydriatic present, and express as per cent. of total alkaloid.

Cats differ considerably in susceptibility to the various alkaloids and, therefore, immediately after receiving a lot of cats for testing purposes their individual susceptibility, to each alkaloid, to be tested, should be determined. A record should be made of the minimum effective concentration of each alkaloid for each cat and used for comparison with the amounts of the unknown required to produce the same effects upon the same cat.

The susceptibility of each cat to the "standard" should be checked at least every two months.

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CHAPTER XII

LOCAL ANESTHETICS

One of the most important duties of the Control Department of a large pharmaceutical manufacturing house is to carefully investigate all complaints received. This is especially true in cases where the physician claims the preparation does not possess the required activity. Such complaints at times make it necessary to check the chemical assay with biologic tests, even though the chemical test is supposed to give a true index to the therapeutic value of the drug or preparation. Many such complaints are received in reference to tablets and ampul solutions of the various local anesthetics. This chapter, therefore, includes the various biologic methods available for measuring the strength of such preparations. All of the methods depend upon the power of the drug to produce sensory paralysis which is evidenced by failure to respond to sensory stimuli. The susceptibility of the various nerve fibres presents marked and characteristic quantitative differences. It is, therefore, advisable to employ more than one method when comparing the activity of an unknown sample with that of a standard.

There are four principal methods available for the biologic standardization of local anesthetics:—

1. Anesthesia of Cornea
2. Anesthetic Action on the Tongue
3. Paralysis of Nerve-fibres on Direct Application
4. Hypodermic Injection into Dogs.

1. **Anesthesia of Cornea.**—This method consists in determining the minimum amount of the drug per c.c. of solution, one drop of which will produce anesthesia of the cornea of a cat or rabbit. In other words the determination of the smallest amount required to abolish the winking reflex when the cornea is touched with a stiff bristle.

Apparatus Necessary for Experiment. 25 c.c. volumetric flasks for making accurate dilutions, selected 1 c.c. Mohr pipettes graduated in 0.01 c.c. with slender tips which deliver exactly 0.05 c.c. per drop and stiff bristle mounted at right angles on a wooden rod.

Animals. Full grown cats or rabbits which are accustomed to being handled.

Preparation of Solutions. Dissolve a representative number of tablets or a sufficient quantity of the granulation or powder in distilled water, to make a solution containing 1 per cent. of cocaine hydrochloride, quinine urea hydrochloride or stovaine, or 2 per cent. of procaine or hydrocyanic acid. Other salts in sufficient concentration to approximate the activity of the above solutions.

Method of Administration. The cat is held on its side on the table. While an assistant holds its four legs the operator drops 0.05 c.c. (1 drop) of the solution into the outer margin of the eye. One hand is used to hold the head and compress the inner canthus while the other hand is used to open and close the lids until the fluid has just apparently disappeared.

Actual Standardization. First touch the cornea of each eye with the stiff bristle and note the winking reflex. Then apply 0.05 c.c. (one drop) of the "standard" solution in one eye and one drop of the solution of the unknown in the other eye. Test the reflex of each eye at intervals and note the time when anesthesia appears and disappears. The strength of the standard or unknown solution should then be adjusted until equal amounts (0.05 c.c.) produce complete anesthesia in the same length of time and the action is of about the same duration. The relative activity of the two preparations is then determined by comparing the drug strength of the two dilutions. Both the "standard" and "unknown" solution should be tested upon the same animal.

Variations may further be avoided by constantly changing the order of the applications so that the same solution will not be applied to the same eye twice in succession. In other words, in the first test the unknown solution should be applied to the right eye and the standard solution to the left eye. In the next test the unknown solu-

tion should be applied to the left eye and the standard to the right eye, etc.

Although different animals vary in susceptibility this does not influence the test as both standard and unknown are tested upon the same animal.

A more delicate test is to determine the **pain-reaction of the human cornea.** For this purpose a series of straight hairs of different diameters are employed. The hairs should be one or two inches long and cemented to the ends of small wooden sticks. Hairs of about five different diameters should be used. After applying the solution of the drug the hair of the smallest diameter should be pressed against the cornea with sufficient force to just bend the hair. If the eye is not resistive to this force the next larger diameter hair is applied, etc., until the pain is felt. The concentration of the solution must be adjusted until it is of such strength that the minimum pain sensation is produced by one of the series of hairs.

It is possible to express the force exerted by the different hairs by pressing them against one pan of a balance and counterbalancing with weights. The weight is divided by the square area of the cross-section of the hair, calculated from its micrometer measurement.

2. **Anesthetic Action on the Tongue.**—This method is not as accurate as the other methods but serves as a rough quantitative test. The method consists in determining the weakest solution which will anesthetize the tip of the tongue.

Apparatus Necessary for Experiment.—Volumetric flasks and pipettes for making accurate dilutions and small filter papers.

Preparation of Solutions.—Same as under "Anesthesia of Cornea" p. 164.

Preparation of Experiment.—Before undertaking to test the activity of a solution of unknown strength, the operator should determine the weakest solution of the "standard" required to produce loss of sensibility to touch within five to ten minutes.

Method of Administration.—A small piece of filter paper is saturated with the solution and placed on the tip of the tongue.

Standardization.—A solution of the sample to be tested should be made of the same drug strength as that of the minimum effective

solution of the "standard." This is applied to the tongue and the sensibility to touch noted after five and ten minutes. Depending upon the results of this preliminary test the strength of the unknown solution is increased or decreased until it produces the same effects as the standard within the same time limits. The relative strength of the two preparations is then determined from the drug strength of the two solutions.

3. **Paralysis of Nerve-fibers on Direct Application.**—This method consists in determining the smallest amount of the preparation required to produce complete anesthesia of the sciatic. This method, therefore, really measures the depression of the motor-fibres, which are more resistant than the sensory fibres. However, since the two are generally parallel this is in fact an indirect measure of anesthetic power.

Apparatus Necessary for Experiment.—Moist chamber, inductorium, light muscle lever, two dry cells, bell wire, single key, small camels-hair brush, volumetric flasks, pipettes, scalpel, probe, small forceps and scissors.

Animals.—Frogs weighing 40 to 60 grams.

Preparation of Solutions.—Same as under "Anesthesia of Cornea" p. 164.

Preparation of Experiment.—Make two muscle-nerve preparations with long nerves; attack each to the femur clamp in a moist-chamber and to muscle lever; arrange dry cells, single key and inductorium for stimulation of the end of the sciatic nerve. Determine the threshold stimulus of the sciatic.

Standardization.—After determining the threshold stimulus of the sciatic for each muscle-nerve preparation paint a short stretch of the one nerve with the "standard" solution and the other with the solution of the "unknown." Again determine the threshold at five minute intervals until anesthesia is complete. Depending upon the results of this preliminary test the strength of the unknown solution is increased or decreased and again tested upon new muscle-nerve preparations until it produces complete anesthesia within the same period of time as that required by the standard solution. The relative strength of the preparations is then in inverse proportion to the drug strength of the two solutions.

4. **Hypodermic Injection into Dogs.**—This method was devised by the author[1] especially for checking the chemical assay of tablets and tablet combinations containing cocaine, procaine and other local anesthetics. The method consists in determining the minimum amount per c.c. required to produce complete local anesthesia in dogs, within five minutes, when injected subcutaneously. The absence of sensitiveness is proven by touching the site of injection with a red hot wire loop.

Apparatus Necessary for Experiment.—Accurately graduated hypodermic syringe, volumetric flasks, wire loop about 3 mm. in diameter made from about 20 gauge wire and a pair of a curved scissors.

Animals.—Short haired medium sized dogs.

Preparation of Solutions.—The solutions for injection should be of such strength that the volume injected is approximately 1 c.c.

Preparation of Experiment.—With the curved scissors closely clip the hair from about one square inch of the dog's back in a series of spots about four inches apart and two inches on either side and parallel to the back bone.

Method of Injection.—The solution should be injected with an accurately graduated syringe in such a way that the *point* of the needle is in the *center* of the spot from which the hair was clipped.

Actual Standardization.—Inject 1 c.c. of the "standard" solution directly beneath the center of one spot and 1 c.c. of the solution of unknown strength under another spot. After exactly five minutes touch the center of each spot with the red-hot wire loop and note whether the animal shows any signs of pain or irritation. Basing the dosage upon these results other series of injections are given, progressively increasing or decreasing the strength of the unknown and standard solution until the weakest dilution of each is found, one c.c. of which will produce complete local anesthesia (absence of sensitiveness to red hot wire loop). The relative activity of the two solutions is inverse to the drug strength of the two solutions.

[1] "The Biologic Standardization of Local Anesthetic" by Paul S. Pittenger, Jour. A. Ph. A., 10, 1921, 746.

"The Biologic Standardization of Local Anesthetics" Continuation of a Previously Reported Paper," by Paul S. Pittenger, Jour. A. Ph. A., 12, 1923, 239.

As dogs vary markedly as to susceptibility both the known and unknown solutions should be tested upon the same animal.

A marked variation is also found in the thickness of dogs' skin and consequently in their sensitiveness to the red hot wire loop. Only dogs which show a marked sensitiveness to the hot wire loop should be used.

Although the quantity varies with different dogs the average minimum amount of *Cocaine* that will produce local anesthesia in a limited area is 0.006 gm. (1 c.c. of a 0.6 per cent. solution), or 0.07 gm. (1 c.c. of a 7 per cent. solution) of Procaine.

With properly selected dogs this method is sensitive to a variation 0.1 c.c. of solutions of the above strength.

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CHAPTER XIII

VITAMINS

Until recently food values were classified upon the four great nutritional elements: fats, carbohydrates, proteins and mineral salts. It has been shown by various research workers, however, that certain substances known as "food accessories," "unidentifiable dietary factors," "growth promoting substances" or vitamins are of great importance in nutrition. These food accessories are six in number and are known as Vitamin A, Vitamin B, Vitamin C, Vitamin D, Vitamin E and the P-P Factor.

The exact character of these substances has not been established but the most recent work on their chemistry indicates that the water soluble vitamins are amino compounds, while the fat soluble vitamins resemble Cholesterol in composition.

If the diet is deficient in any one of the six vitamins listed above the following "Deficiency Diseases" will result:

1. Ophthalmia, or Xerophthalmia, due to lack of vitamin A.
2. Beri-beri due to lack of vitamin B.
3. Scurvy, due to lack of vitamin C.
4. Rickets, due to lack of vitamin D.
5. Sterility disease, due to lack of vitamin E.
6. Pellagra, due to lack of "Factor P-P."

Vitamin A, is one of the growth promoting vitamins, usually called *Fat Soluble A* because of its principal origin.

The main source of vitamin A is cod liver oil. It is also especially abundant in butter, egg yolk and cream. Other sources are glandular tissues of fowls and food animals, carrots, spinach, lettuce, alfalfa, string beans, whole wheat bread, raw cabbage, celery, cheese, dandelion, ice icream, orange oil, peaches, fresh peas, pineapple, tomato and wheat.

This vitamin is relatively stable as it is present in the yolks of hard boiled eggs. In the absence of air it may be heated to relatively

high temperatures but in the presence of air or oxygen it is readily oxidized.

Vitamin B, is also a growth promoting vitamin referred to as *Water Soluble B* and is sometimes called an *antineuritic vitamin*.

The main source of vitamin B is yeast. It is also especially abundant in asparagus, beans, spinach, tomato and wheat. Other sources of supply include practically all natural foodstuffs principal of which are apples, whole barley, whole wheat bread, buttermilk, cabbage, cauliflower, celery, yellow corn, dandelion, egg yolk, whole wheat flour, grapefruit, ice cream, lemon juice, lettuce, milk, oats, onions, orange juice, parsnips, peanuts, peas, pineapple, potatoes (sweet and white), prunes, rice (whole grain), turnip, walnut and whole wheat.

Vitamin B is more stable than either vitamin A or C. It will stand the temperature of ordinary canning and cooking so that these procedures do not destroy this vitamin to any marked degree. It also has a marked stimulating effect upon the appetite.

Vitamin C, is referred to as "*Water Soluble C*," or the *antiscorbutic vitamin*.

The main source of vitamin C is raw cabbage, lemon juice, lettuce, orange juice, fresh peas, pineapple, raspberries, raw spinach, tomato and turnip. Other sources are apples, bananas, raw beans, carrots, celery, grapefruit, milk, onions, peaches, potato (sweet and white) and strawberry juice.

Vitamin C is the most unstable of the vitamins as it is readily destroyed by high temperatures and oxidation. It is not as readily destroyed in the absence of oxygen. This is proven by the fact that cooking in open kettles is more destructive than modern canning procedures.

Vitamin D, stimulates the growth of yeast and probably other microörganisms and possesses antirachitic properties. It is, therefore, called the *antirachitic vitamin*.

The main source of vitamin D is cod liver oil. It is also comparatively abundant in milk and egg yolk. It is found in lesser amounts in bread made with milk, butter, dandelion, eggs and lettuce.

Vitamin E, is fat soluble and essential to the reproduction of the individual and hence has been called the *fertility vitamin, reproductive*

vitamin, or *anti-sterility factor*. It was originally known as *vitamin X*.

The main source of vitamin E is the oil of the wheat germ. It is also comparatively abundant in hemp seed, legumes (germinated), and wheat grain. Other sources are alfalfa, beans, cod liver oil, yellow corn, dandelion, eggs, whole wheat flour, lettuce, meats, oats, and olive oil.

Vitamin E is stable to heat, light and air.

The P-P Factor, or *pellagra-preventive* substance is the name given to the substance which prevents or cures pellagra.

The main source of the P-P Factor is Yeast. Other sources are lean meat, butter, carrots, eggs, lettuce and spinach.

Biologic Assays for Vitamin and Standardization of Vitamin Containing Products

The scant knowledge of the chemical composition of the various vitamins renders it impossible to quantitatively measure by chemical means the vitamin content of a given product.

Color reactions for the identification of the different vitamins have been proposed. Of these color reactions the following are apparently the most useful:

Vitamin A.—Rosenheim-Drummond's Test.[1] Fearon's Test.[2]

Vitamin B.—Jendrassik's Test.[3]

Vitamin C.—Bezssonov's Test.[4]

Vitamin D.—Shear's Test.[5]

Although these color reactions are an aid in the identification of the various vitamins, *they have not been sufficiently developed to be dependable for quantitative assays.*

The biologic assays for vitamins, however, have been sufficiently developed to render it possible to *quantitatively* determine the vitamin content of a medicinal or food product.

[1] Rosenheim and Drummond: Biochem. Jour. 19, 753, 1925.

[2] Fearon: Biochem. Jour. 19, 888, 1925.

[3] Jendrassik: Jour. Biol. Chem., 57, 129, 1923.

[4] Bezssnov: Compt. rend., 173, 466, 1921; Biochem. Jour., 18, 384, 1924.

[5] Shear: Proc. Expt. Biol. Med., 23, 546, 1926.

The details of these assay processes follow:

General Principles

Before considering individual vitamin assays the bio-assayist must be thoroughly familiar with important general principles which must be applied to all assays of this type.

Cages.– Cages for vitamin assays and other nutrition experiments should be made of metal, provided with false bottoms and so constructed that they may easily be cleaned and sterilized. The false bottoms are essential or otherwise there is a possibility of the animals re-ingesting waste food, with resultant vitamin conservation, and retarded symptoms of vitamin starvation.

A satisfactory cage devised by the author is shown in Figs. 67 and 68.

The sides of the cages are constructed of 1/4″ mesh galvanized wire netting 8″ × 28″. The ends are lapped about 1″ and fastened together with brass split-rivets and washers thus making a cage 8″ high and 8 1/2″ in diameter. (Fig. 68*D*.) The lid (Fig. 68*C*) is a galvanized 9″ and the bottom (Fig. 68.*A*) a galvanized 10″ jelly cake tin. The false bottom is made from a 9″ jelly cake tin of the loose bottom type. The tins for this purpose are purchased *without bottoms*. A bottom of 3/16″ galvanized wire netting is then soldered into these tins (Fig. 68*B*). Figure 68*E*, shows the all-glass drinking fountain and Fig. 68*G*, the galvanized iron hanger for the fountain which is bolted to the outside of the cage. The cake tins should be heavily galvanized. Figure 68*F*, shows the Parson's feeding cup which is provided with hooks so it may be hung at any desired heighth on the inside of the cage.

The cage is assembled as follows: The cake tin *B*, with the wire netting bottom is placed in an inverted position inside of the larger cake tin *A*. This forms the false bottom as it leaves a space of approximately 1″ between the wire netting of *B* and the bottom of *A*. The bottom of *A* is covered with 2 or 3 sheets of 185 mm. filter paper to absorb water and urine. The side of the cage *D* is then placed over the inverted tin *D* and the lid *C* placed on top. A hole is made in the side of the cage of sufficient size to allow the

end of the drinking fountain to pass through. Bedding must not be used.

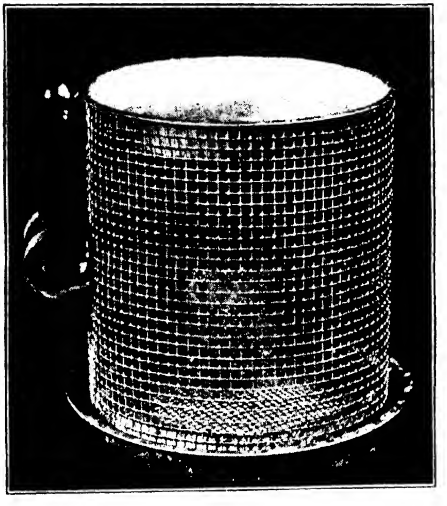

FIG. 67.—Author's cage for vitamin assays, assembled.

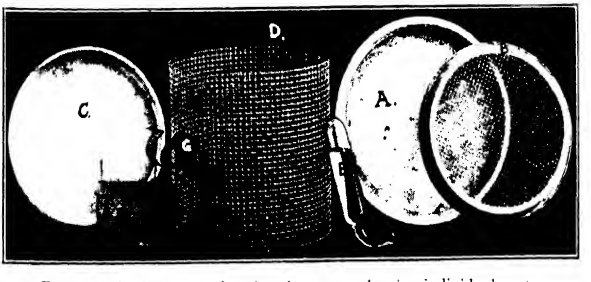

FIG. 68.—Author's cage for vitamin assays, showing individual parts.

Cages must be cleaned at least weekly and sterilized at least every two weeks.

When experiments are being conducted on more than one vitamin at a time great care must be exercised while cleaning cages to prevent carrying vitamins from one set of cages to another. Attendants must be instructed to thoroughly clean all brushes, sinks, hands, etc., after handling one set of cages before starting on another.

Housing Rats during Metabolism Experiments.—Rats should be kept in a room at about 72 to 76° F. During metabolism experiments the temperature should be kept as nearly constant as possible. Drafts should be avoided. In order to maintain a uniform temperature and to avoid drafts practically all breeders and many experimenters prefer the wooden cage. Wooden cages, however, although satisfactory for breeding purposes are not satisfactory where it is necessary to keep dozens or hundreds of rats in individual cages. They are too bulky and difficult to clean and sterilize. On the other hand rats kept in cages as shown in Fig. 67 without bedding are affected by drafts and the failure of the cage to retain any of the body heat of the animal. In order to combine as much as possible the advantages as to size, cleanliness and ready sterilization of the metal cage with the draft preventing and heat retaining qualities of the wooden cage the author has devised special shelves for holding the metal cages.

The shelves shown in Fig. 69 are constructed of 7/8″ lumber. Each shelf measures 1′ × 6′ × 9″ high and accommodates six cages. The space between the shelves is only 3/4″ more than the height of the assembled cage which makes it impossible for the adult rats to lift the cage lid sufficient to escape. This eliminates the necessity of attaching the lids to the cages which greatly facilitates handling and cleaning. The shelves are provided with hinged wooden fronts which extend to within 2″ of the top of each shelf. When these fronts are closed the rats have practically all of the advantages of being housed in wooden cages.

In addition the hinged wooden fronts greatly facilitate the feeding of the daily dose of product under test. The fronts are so hinged that when opened the bottom edge of the front butts against the front edge of the shelf, thus acting as an extension to the width of the shelf. In feeding experiments the front of one shelf is lowered at a time and the cages slid forward as shown on the left hand side

of Fig. 69. By use of the step ladder shown it is possible for one operator to hold a pipette for measuring doses in one hand, lift the lid with the other and pipette the desired dose directly into the small feeding cup. The cages are then slid back in position and the front

FIG. 69.—Shelves for holding metal metabolism cages. The hinged wooden fronts when closed protect against drafts and when open facilitate feeding, etc. (Photograph from Author's Research Laboratory, Sharp & Dohme.)

closed. The daily doses may thus be rapidly given without lifting the cages from the shelves.

Breeding and Exercising Cages.—See Care of Animals Chapter XVII.

Feeding Cups.—Rats scatter a large percentage of the food placed in their cages unless special feeding cups are provided. Scattered

food which has passed through the false bottom will absorb urine, become mixed with droppings and thus contain excreted vitamins. *Such food must be discarded.* As the preparation of vitamin-free foods is very expensive it is essential to employ feeding cups which prevent scattering.

There are many types of cups specially designed for this purpose. The author prefers the cup designed by H. T. Parsons and N. Alezandria described in the Jour. of Lab. and Clinical Med., May, 1924. (See Fig. 70.)

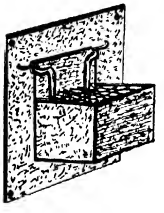

Fig. 70.—Parsons Feeding Cup.

This cup is provided with a wire screen which is placed on top of the feed. The screen is held in position by two wires which pass from the support at the rear, above the cup, through the openings in the screen, to the bottom of the cup.

In addition to the above feeding cups which contain the basal diet small cups are required for holding the daily dose of the product under test. The small glass cups used for placing upon the floor under the casters on furniture are very handy for this purpose. They are heavy, shallow, have all rounded edges and are hard to upset. The doses may be pipetted or weighed directly into these cups. They may be easily cleaned and sterilized.

Drinking Water.—In considering a feeding experiment with known or unknown vitamin substances or one with vitamin-free food, it is assumed that caged animals will be used and upon the supposition that these animals will get only what the experimentor provides for them and actually gives to them.

This is one of the greatest fallacies in feeding work and must be overcome before the investigator will meet with uniform success. Before considering the diet at all, the kind of drinking water, its origin and composition must be known.

Drinking water may contain extraneous mineral salts, bacteria, algae, wild yeasts and vitamin, which will entirely upset all other calculations and spoil the tests.

Distilled water is the only drinking water which will uniformly give reliable results, especially in hot weather, and which is comparable with the best work of the best workers.

A stock solution of iodine 5 grams, potassium iodide 10 grams and distilled water 100 grams should be kept on hand and two drops of this solution added to each litre of distilled water used for drinking purposes.

The most satisfactory method of supplying water is by use of the glass drinking cup. (See Fig. 71 and Fig. 68*E*.) The cups should be hung in such a position as to be easily reached but difficult to soil the water.

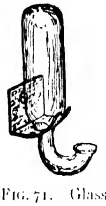

Fig. 71. Glass Drinking Cup.

Many of the foods used for vitamin assays contain fats. This grease adheres to the glass and, therefore, great care should be exercised to prevent the carrying of vitamins from one set of experiments to another by means of careless exchange of drinking cups. If the cups are filled from a pan containing the distilled water, a separate pan should be used for filling the drinking cups for *each* experiment or the pan should be thoroughly cleaned after watering each set.

Cleanliness and Sanitation. - While the animals may be caged; the insects, larvae, bacteria and even small rodents are not caged and many times enter the cages of the test animals in large numbers, without knowledge or notice of the analyst, and wholly vitiate or disqualify the results, from a vitamin standpoint.

Experimental rats will consume many small insects; while test chicks, chickens or pigeons will consume even more; and larger experimental animals will devour an occasional small rodent, with sufficient food and vitamin effect to distort growth curves for days or even weeks.

The only remedy for this is *screens*.

Furthermore, with due regard to pure water and cleanliness, as above, you will find that what you called pure water when given, soon becomes a mixture of water and other food available, whatever the test animal may be.

The feeding test, with warm-blooded animals, is usually in a rather warm room and this food-water mix quickly becomes infected with bacteria and yeasts, and vitamins are synthesized there within a few hours, supplying A, B, C, D, . . . Z, everything adequate for growth, and the symptoms of vitamin deficiency are greatly

distorted. This is the reason for the addition of iodine to the drinking water.

Roughage.—*It is agreed that roughage must be supplied to white rats, upon an artificial food mix of the fatty paste type. For this purpose, Agar is often used by some of the best experimentalists. It has been shown that Agar is not wholly inert as a factor, since it swells to great spongy, wet mass in the intestine, thus increasing the food surface area for bacterial growth, with increased putrefactive absorption and an increased output of indican in the urine. This is very important in vitamin research.*

PREPARATION OF THE EXPERIMENTAL FOOD

The Protein.—The preparation of the complete food for the Norwegian white rat—with all vitamins included or with any one *omitted*, is typical in character and will serve the purposes of this short article.

Such a food must include an adequate protein, containing a full quota of amino acids, necessary for growth, and the protein in most wide use is the Protein Casein, free from the albumin of milk, free from salts, fats and other extraneous matter of milk, which would confuse or totally spoil the experiment.

To prepare *pure casein*, it is essential to begin with skim milk, curdle with weak acid to the exact separation point, filter, remove all water-soluble solids, wash curd thoroughly, redissolve curd in least amount of sodium hydroxide (excess of caustic soda will invalidate the casein), filter absolutely clear the sodium caseinate solution, reprecipitate with dilute acetic acid, filter, wash for days with dilute acetic and dilute hydrochloric acid, wash with cold pipe water, distilled water, strong alcohol, absolute alcohol, absolute ether. Filter press, air dry and grind fine. Standardize by feeding daily amounts, in adequate quantity, by the white rat method and analyze chemically when the salts of calcium or other bases interfere with the nature of the study.

Too often, *crude casein* or "mixed proteins of milk" are used as the nitrogenous element of the diet, which introduces an unknown factor at the outset of the experiment.

Since the sodium salt of the protein casein is soluble in cold water, it is considered that no preparation of this protein can be admitted

as pure, unless its sodium solution has been filtered perfectly clear before final precipitation.

The selection of the *protein* is of the utmost importance. It must be adequate in its molecular make-up for body-growth, free from vitamins and, many times, free from mineral salts, especially calcium.

The wide use of "crude, commercial casein," a by-product of cheese factories, is an unscientific practice and leads to much confusion in interpretation of results. A pure preparation of casein cannot be prepared from a dry, crude preparation, since the solubility of the albumin has been altered and the mineral salts have become combined with the water-soluble protein.

A pure preparation must be prepared directly and continuously from fresh milk and the clear, filtered solution of sodium caseinate must be prepared.

From our own studies, we have found it more practical and economical to use "Casein-Harris," prepared under the personal direction of Dr. Isaac F. Harris, who had a wide experience in the protein laboratories of Drs. Osborne and Mendel, Yale University.

The Carbohydrate.– Corn starch is most commonly used to supply calories in this form, and while the usual commercial grades are free from Vitamins A and B, it is not safe to use any starch without running a blank test for these vitamins, or one should purchase a starch which has been tested by others and offered with a guarantee that it is free from vitamins.

The Fats.– Most diets for the white rat contain high percentage of vitamin-free fats. The investigator has a wide range of choice of these vitamin-free fats, but it is never safe to assume that a given fat is free from Vitamin-A or any other vitamin, until it has been proven to be blank in these factors, by actual feeding experiment in the hands of the experimentalist or before it was obtained by him.

The Mineral Salts.– While it is fairly safe to assume the freedom of most mineral salts from all vitamins; this, likewise, should be proven by the experimental method. The greater danger here is the failure to obtain the correct list of salts and the greater difficulty of obtaining an absolutely fine and homogenous powder, uniform throughout in its make-up of water-soluble and insoluble salts. Obviously, if any portion of a salt mix is different in composition

from any other portion, it will show up in the effects of its use throughout a colony of rats and can wholly distort the resulting experiments.

The Vitamin Accessories. *Vitamin A (and D).*—Cod Liver Oil is entirely satisfactory as a source of these Vitamins A and D in a fatty, food mix for the white rat.

The percentage of the oil required is small, it is easy to distribute through the food mix, is palatable to the animals, is abundant, available and cheap. Certain precautions are, however, necessary.

One should purchase a *brand* of cod liver oil of known origin and known to be potent in these vitamins. It is not necessary, usually, to know the exact vitamin value of such oil, because a large surplus exists there for these purposes, but one must know that he has cod liver oil of high purity and not some "fish oil" or adulterated specimen. Tested brands are available and these should be obtained.

A fractional preparation or concentrate of cod liver oil may be used as a source of Vitamins A and D or sometimes only containing Vitamin D—which can be determined in each case. Or, butter fat will provide Vitamin A, though it must be used in higher percentage than cod liver oil, and this calculation must be made.

Although in the absence of air or oxygen vitamin A may be heated to relatively high temperatures a number of investigators have shown that when air or oxygen is present Vitamin A oxidizes quite readily; hence, the necessity of a "check-up" on this value when substitutes or concentrates of cod liver oil are used. Cod liver oil and butter fat are quite permanent in Vitamin A, when left alone, but when the particles are separated, and exposed to oxidation in a dry food mix, this effect takes place more readily. Many derivatives of cod liver oil and butter fat oxidize more rapidly.

It is advisable, therefore, to prepare diets containing these vitamins at frequent intervals and to store them in closed containers away from excessive heat or sunlight.

Vitamin B.—The accessory vitamin B may be added in the form of any substance which contains it in known quantity, but the most concentrated and uniform source and most widely used is a freshly tested, dried, *brewers' yeast* of a known concentrate or fractional preparation from it. Bakers' yeast contains less vitamin B than a

good grade of brewers' yeast and very commonly contains an excess of ammonium salts, employed in its culture medium. Also, bakers' yeast of commerce either comes in the form of the moist cake, containing unknown water and dry starch, or it comes as a dry cake or powder, commonly adulterated with other substances, not deleterious for the baking trade, but out of place in a scientific feeding experiment.

Dried, pasteurized or killed cultures of pure brewers' yeast contain the largest proportion of vitamin B available in any raw, commonly occurring food substance. When one wishes to refer to the exact amount of vitamin B of the diet, it is important to use *killed cultures* of brewers' yeast, otherwise the large number of living yeast cells fed, may multiply somewhere in the digestive tract, with synthesis of vitamin B, in unknown amount which cannot be computed in terms of food intake or physiological effect. The synthesis of vitamin B in the intestinal tract by the bacterial flora is beyond control, but the addition of living yeast cells is avoidable.

Vitamin Concentrate.—The best, concentrated preparation of vitamin-B with which the author is familiar, and employs in his own experiments, is the Osborne and Wakeman fraction from brewers' yeast, described in Journal Biological Chemistry, December 1919. This is now a commercial preparation, made by the Harris Laboratories, Tuckahoe, N. Y. and is available for research as Yeast Vitamin-Harris. Another advantage in the use of the Osborne and Wakman fraction is that it contains no coagulable protein, a minimum of extraneous nitrogen and no nucleo protein compounds of whole yeast cells, which is of great importance whenever the intake of the kind or the quantity of the protein is a factor.

Preparation for the Feeding Experiment.—When the investigator has duly considered all these factors and has arranged to keep the animals, cages, food cups and everything else clean, bacterially clean, and has mastered the physiology of the experimental animal under *normal feeding*, as a control—then and only then is he ready and qualified to plan an exact feeding experiment, to decide upon the diet, the housing, the weighing and measurements, to plot body weight curves and to compare radiographs of the test animals with those of the normal, with any hope of arrival at the proper conclusion.

BIOLOGIC ASSAY FOR VITAMIN A

This method consists in determining the minimal amount of the preparation under test necessary to produce growth resumption and cure other induced symptoms of vitamin A starvation in young albino rats.

Apparatus Necessary for Experiment.—Individual cages, pipettes, scales sensitive to 0.5 gm., weights ranging from 1 to 500 gms. and chart paper for plotting weight curves.

Animals.—Young albino rats not less than twenty-five days and not more than twenty-nine days old, and weighing not less than 35 gms., and not more than 45 gms. The rats must be of a constant source and bred preferably under the control of the experimentor.

Rats for U.S.P. Cod Liver Oil Assays must be fed a diet of the following composition:

Finely ground whole wheat	66 per cent.
Whole milk powder	33 per cent.
Sodium chloride	1 per cent.

The antirachitic requirements of the breeders shall be assured.

Preparation of Experiment.—The number of rats to be placed on the vitamin A free diet depends upon the nature of the product to be assayed and the operators knowledge as to its approximate vitamin content. The average assay requires about 24 rats. Four for controls and five series of four rats each to be given varying daily doses of the product. For example, if the product under consideration is a liquid preparation supposed to contain vitamin A, the assay would be conducted as follows:

Take twenty-four rats which meet the requirements specified under *animals*, place in individuals cages and feed a vitamin A free basal diet of the following composition:

Casein, or desiccated fresh meat, rendered free from vitamin A	18 per cent.
Salt Mixture, such as that of Osborne & Mendel[1] or McCullum and Davis[2]	4 per cent.
Starch, sufficient to make	100 per cent.

[1] Osborne and Mendel: Jour. Biol. Chem., Vol. 15, p. 317, 1913.

[2] McCullum and Simmonds: Jour. Biol. Chem., Vol. 33, p. 55, 1918.

Brewers Yeast sufficient to meet the vitamin B requirements of the animal (6 grams per 100 grams of food), mixing it with the basal diet during the vitamin A free period.

FIG. 72. Rat showing typical symptoms of Vitamin A Deficiency. Note shaggy hair, hunched back and xerophthalmia.

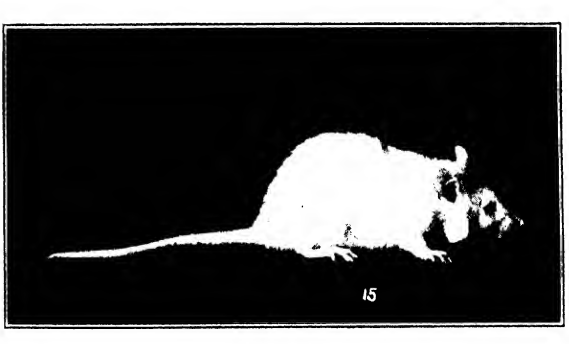

FIG. 73.—Rat shown in Fig. 72 after receiving Vitamin A.

Weigh each rat and plot weight curve every three to five days. (See Figs. 74*B* and 74*A*.)

After the rats are placed upon the above basal diet they will continue to grow until their store of vitamin A is exhausted. This usually requires from 4 to 7 weeks after which is some cases the

animal remains at a constant weight for a short period and then begins to loose weight, while in other cases the loss in weight immediately follows the increase.

The rats are continued upon the vitamin A free basal diet until the weight remains stationary or declines for at least seven days and the animal shows the typical symptoms of vitamin A deficiency, *i.e.*, rough and shaggy hair, knotty tail, hunched back, ophthalmia or xerophthalmia. (See Fig. 72.)

The length of time required for the rats to commence to loose weight and show these symptoms varies considerably. Therefore, all the rats used for an assay should not be started on the vitamin containing food at the same time. Each rat is an individual study and should not be given the vitamin containing food until it reaches the above condition. Likewise all of the animals that reach this condition first should not be placed in the same series. If six animals start to loose weight at approximately the same time, one should be placed in each of the six series and of the next six to loose are placed in each series, etc., until all the animals except the controls are receiving the preparation under test.

Preparation of Solutions.—The preparation to be tested should be diluted sufficiently to be accurately measured. Emulsions etc., may be diluted with distilled water. Cod Liver Oil, butter etc., should be diluted with a known vitamin A free cottonseed oil. How much to dilute the product is based upon the operators knowledge or idea of its approximate strength.

Method of Administration.—Small doses may be pipetted directly into the rats mouth. Larger doses are measured into the small cups previously described.

Actual Standardization.—After not less than seven days of stationary or declining weight, divide the twenty-four rats into six series of four rats each. Continue the rats of the first series on the basal diet as controls. To each rat of the second series, in addition to the basal diet, administer 0.25 c.c. per day of the product under test. To each rat of the third series administer 0.5 c.c., to the fourth series 1.0 c.c., to the fifth series 2.5 c.c. and to the sixth series 5.0 c.c. per day.

The test period should continue for thirty five days. During this time all the rats of the control series should continue to loose weight

and finally die, thus proving the absence of vitamin A in the basal diet. If the preparation has been properly diluted the rats receiving the small doses will continue to loose weight and die, those receiving the next larger size dose will either remain at practically constant weight or slowly increase or decrease in weight. The series receiving still larger doses should show a more rapid weight increase. (See Fig. 74*B*.) The object of the assay is to determine the *minimum* amount of the preparation which will produce growth resumption.

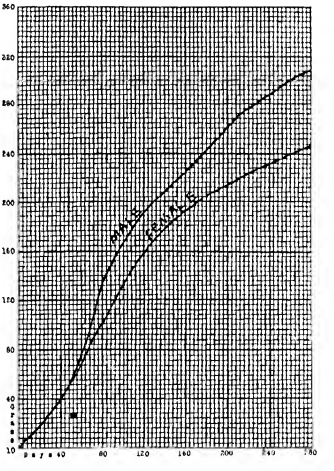

FIG. 74A. - Curve showing average normal growth of Albino Rats.

If all of the rats continue to die the test must be repeated using larger doses or a stronger solution. If all of the rats show growth resumption and the symptoms of the vitamin A deficiency are cured the test must be repeated using a more dilute solution of the product under test.

Standards.—The U.S.P. *X* contains the following optional standard for Cod Liver Oil.

"The Vitamin A potency of Cod Liver Oil shall be expressed in units per gram of oil, the unit to be the minimum daily amount of

Cod Liver Oil required to cure induced symptoms of Vitamin A starvation in young albino rats, and to cause a gain in weight of from ten to twenty grams within a period of thirty-five days under the conditions of growth and diet specified in this assay."

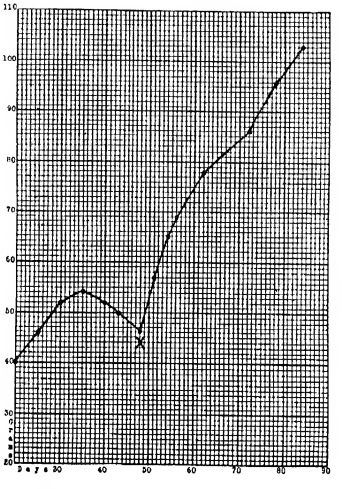

FIG. 74*B*.—Typical growth curve, Vitamin A assay. At X material containing vitamin A was added to daily diet.

"No oil shall be labeled as assayed by the U.S.P. method unless it contains at least 50 units per gram of Oil."

BIOLOGIC ASSAY FOR VITAMIN B

This method consists in determining the minimal amount of the preparation under test that will produce growth resumption and cure other induced symptoms of Vitamin B starvation in albino rats.

Apparatus Necessary for Experiment.—Same as Vitamin A assay.

Animals. Young albino rats weighing 60 to 70 grams. Rats should be of constant source, bred preferably under the control of the experimentor.

Preparation of Experiment. –Place 24 rats of the above description on a Vitamin B free basal diet of the following composition:

Casein	20 per cent.
Butter fat	15 per cent.
Starch	61 per cent.
Salt mixture	4 per cent.

Add sufficient Crisco to make a hard paste.

Weigh every three days and plot weight curve for each rat until they have lost sufficient weight to show the characteristic symptoms of vitamin B deficiency, knotty tail, ruffled, coarse hair, hunched back, emaciation of the legs, etc. (See Fig. 75.)

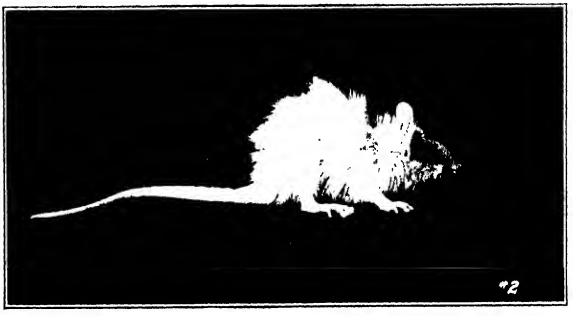

FIG. 75.– Rat showing typical symptoms of Vitamin B deficiency. Note knotty tail, ruffled coarse hair, hunched back, emaciation of legs, etc.

The amount of vitamin B stored in the normal rats body is apparently much less than the amount of A stored. This is shown by the fact that rats placed on a vitamin B deficient diet usually begin to lose weight within ten days. In some cases the loss of weight begins three or four days after the animal is placed upon the B free diet.

The condition and weight curve of each individual rat is studied. Each rat is kept on the vitamin B free food until the growth curve shows a marked loss of weight (see Fig. 78*B*) and the animal has the appearance of the rat shown in Fig. 75.

Preparation of Solutions.– Liquid preparations should be diluted sufficiently to be accurately measured.

Method of Administration. Small doses of liquid preparations are pipetted directly into the rats mouth. Larger doses of liquids are measured into the small feeding cups. Pastes may be weighed in the feeding cups. It is usually best to mix powders in definite proportions directly with the basal diet.

Actual Standardization. After the rats show the typical effects of vitamin B starvation divide them into six series of four rats each. Divide the rats of the first or control series into two sets of two rats each. Continue the one set upon the vitamin B free diet. They should continue to lose weight and finally die. (See Fig. 77*A*.) To

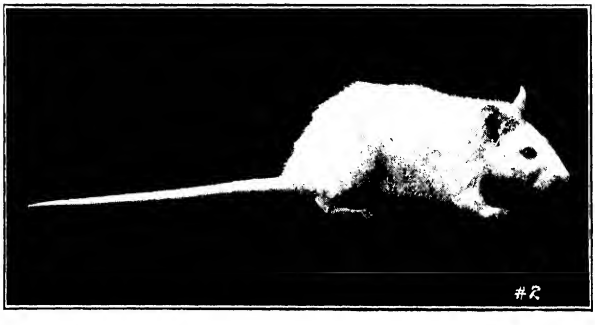

FIG. 76.—Rat shown in Fig. 75 after receiving vitamin B.

the basal diet of the other set add 6 grams of Dried Brewers Yeast (Vitamin B) per 100 grams of food. These rats should immediately respond to the presence of the B vitamin by an immediate and rapid increase in weight. (See Fig. 77*B*.) To each rat of the second series, in addition to the basal diet, administer 0.25 c.c. or grams per day of the product under test. To each rat of the third series administer 0.5 c.c. or grams, to the fourth series administer 1.0 c.c., to the fifth series 2.5 c.c. and to the sixth series 5.0 c.c. per day.

If the preparation has been properly diluted the rats receiving the small dose will continue to lose weight and die (see Fig. 78*A*), those receiving the next larger size dose will either remain at practically constant weight (see Fig. 78*B*) or slowly increase or decrease in

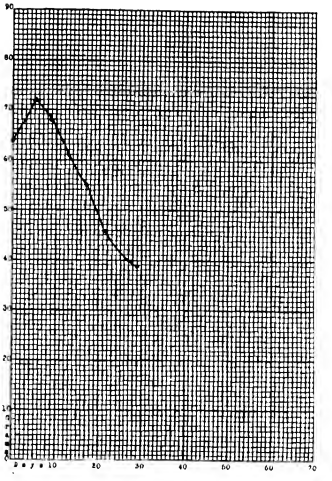

FIG. 77A. Growth curve, Vitamin B assay. Control rat on Vitamin B free basal diet.

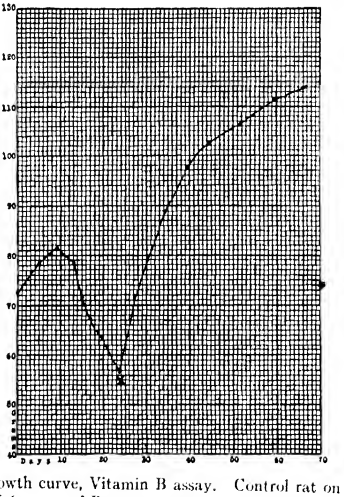

FIG. 77B.—Growth curve, Vitamin B assay. Control rat on Vitamin B free basal diet. At X 6 grams of Brewers yeast was added to each 100 grams of the basal diet.

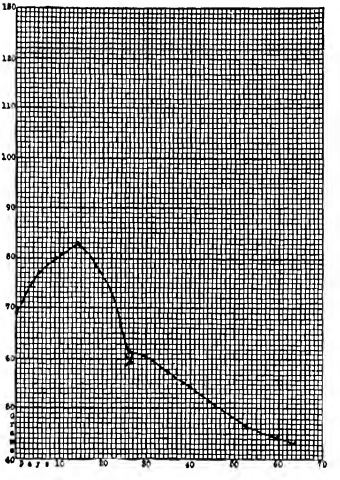

FIG. 78A.—Growth curve, Vitamin B assay. Vitamin B free basal diet. At *X* 0.25 c.c. per day of the Vitamin B containing product was added to the diet.

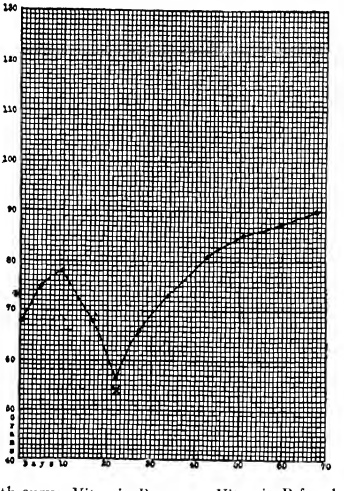

FIG. 78B.—Growth curve, Vitamin B assay. Vitamin B free basal diet. At *X* 100 c. c. per day of the Vitamin B containing product was added to the diet.

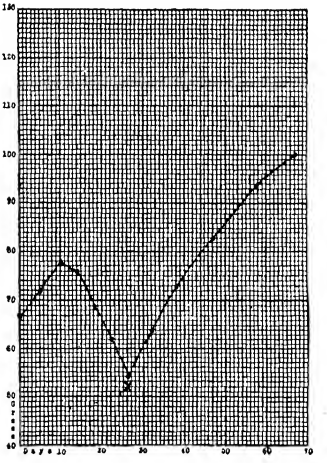

FIG. 79*A*.—Growth curve, Vitamin B assay. Vitamin B free basal diet. At *X* 2.5 c.c. per day of the Vitamin B containing product was added to the diet.

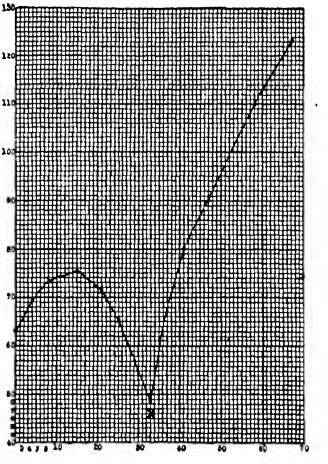

FIG. 79*B*.—Growth curve, Vitamin B assay. Vitamin B free basal diet. At *X* 5.0 c.c. per day of the Vitamin B containing product was added to the diet.

weight. The series receiving still larger doses should show a more rapid weight increase (see Fig. 70.1) and the rats receiving the largest dose should show a still more rapid increase in weight. (See Fig. 70*B*.) If all of the rats continue to lose weight and die the test must be repeated using larger doses. If on the other hand all of the rats show growth resumption and the symptoms of vitamin B deficiency are cured the test must be repeated using a more dilute solution of the product under test.

The accompanying growth curves show that quantitative results may be obtained if the assay is properly conducted.

BIOLOGIC ASSAY FOR VITAMIN C

This method consists in determining the minimum amount of the preparation under test that will cure the symptoms of scurvy in guinea pigs.

Apparatus Necessary.—Properly constructed metal cages with false bottoms, food cups, drinking cups and a hot air oven with temperature control for heating hay.

Animals.—Healthy guinea pigs weighing between 275 and 325 grams.

Preparation of Experiment.—Place ten guinea pigs of the above description on the following vitamin C deficient diet.

Rolled Oats

Hay which has been heated to 110 to 115° C. for three to four hours.

Water

Pasteurized milk (20 c.c. per guinea pig per day).

Guinea pigs that are fed the above diet usually show the typical symptoms of scurvy within two weeks. It will be noted that the animals will wince and cry when handled due to the tenderness of the joints. This tenderness causes them to assume the "scurvy position" (unnatural position such as holding up one tender hind leg) or the so-called "face ache position" (lying with the side of the face resting on the bottom of the cage).

Actual Standardization.—After the guinea pigs show the typical symptoms of scurvy, divide them into five series of two guinea pigs

each. Use the first series as controls. Add 5 c.c. of orange juice (which contains Vitamin C) to each pig in addition to the above diet and make record of signs of improvement. To each guinea pig of the second series, in addition to the above basal diet, give 2.5 c.c. of the product under test. To each guinea pig of the third series give 5.0 c.c., to the fourth series 7.5 c.c. and to the fifth series 10.0 c.c. per day.

Examine each animal daily and make record of signs of improvement.

The relative strength of the preparation is determined by the amount required to produce curative effects comparable to a given amount of orange juice.

BIOLOGIC ASSAY FOR VITAMIN D

This method consists in determining the minimum amount of the preparation under test that will cure rickets in albino rats.

Apparatus Necessary for Experiment.—Individual metabolism cages, pipettes, scales, weights, X-ray, photographic plates and developing apparatus, binocular microscope, and dissecting instruments.

Animals.—Healthy young albino rats weighing 50 to 60 grams.

Preparation of Experiment.—Place 24 rats of the above description on the following rachitic diet:[1]

Patent flour	80.9 per cent.
Egg albumin	10.0 per cent.
Butter fat	5.0 per cent,
Salt mixture[2]	4.1 per cent.
KCl	0.85
Na_2CO_3	0.85
$MgCO_3$	0.286
Ca lactate	2.000
Ferric citrate	0.1
KI	0.0002
$MnSO_4$	0.00078
NaF	0.0024
$KAl(SO_4)_2$	0.00024

[1] Poppenheimer, McCann, and Zucker: Jour. Exp. Med., 35, 447, 1922.

[2] *Salt mixture.*

Albino rats that are fed the above diet usually show the typical symptoms of rickets in 20 to 30 days. After this period of time the rats should have difficulty in using their hind legs. Make radiographs of one of the hind legs of each animal (at the knee joint). Continue each rat on the above diet until the radiograph reveals broad light bands of uncalcified cartilage at the ends of the tibias and femurs (characteristic rachitic bone, see Figs. 80.1 and 80*B*).

FIG. 80*A*.

FIG. 80*B*

FIG. 80*A*.—Active rickets. Radiograph of knee joint of rat on rachitic diet for 17 days. Note especially broad bands of uncalcified cartilage near ends of tibia and femur. (From Hawke and Bergeim's "Practical Physiological Chemistry".)

FIG. 80*B*.—Healed rickets. Radiograph of knee joint shown in Fig. 80*A* after the rat had been fed cod liver oil concentrate 14 days. (From Hawke and Bergeim's "Practical Physiological Chemistry.")

Preparation of Solutions, and Method of Administration. Same as for Vitamin A.

Actual Standardization.—The twenty-four rats are divided into six series of four rats each. As soon as the radiograph of each rat shows the typical rachitic bone, place it in one of the six series. Divide the rats of the first or control series into two sets of two rats each. Continue the one set upon the rachitic diet. Give each rat of the other set 0.25 c.c. per day of cod liver oil in addition to the rachitic diet. To each rat of the second series in addition to the

rachitic diet administer 0.25 c.c. or gm. per day of the product under test. To each rat of the third series administer 0.5 c.c. or gm., to the fourth series 1.0 c.c. to the fifth series 2.5 c.c. and to the sixth series 5.0 c.c. per day.

Repeat the X-ray examinations at weekly intervals. The control rats which are continued on the rachitic diet (first set of group 1) should show more pronounced rickets as time passes with a wider decalcified band. The second set of the control rats (those receiving cod liver oil) should show a gradual recovery from the lameness and as the rickets heal the band of uncalcified cartilage becomes narrower and finally disappears. The experiment should, therefore, be continued until the bone assumes the appearance of a normal bone.

If the preparation has been properly diluted the rats receiving the small doses will show more and more pronounced rickets. Those receiving the next larger size dose will either remain in practically the same condition, show slow signs of healing or slowly show more pronouced rickets. The series receiving still larger doses show more rapid healing and the rats receiving the largest doses should heal still more rapidly.

If all the rats continue to show more pronounced rickets the test must be repeated using larger doses. If all of the rats show rapid healing the test should be repeated using a more dilute solution of the product under test.

The comparative strength of two preparations is in inverse proportion to the daily amounts required to produce complete healing within approximately the same length of time.

The so-called **"Line test"**[1] may be used as a check on the radiographs.

In this test the provisional zone of calcification is brought out by means of a sliver staining method. As the cartilage and metaphysis on either side of the zone of calcification are calcium free, the zone of calcification may be clearly outlined by staining the calcium salts which it contains.

Method.—Dissect out and immerse the tibia in acetone for two days, wash with distilled water and cut in half longitudinally.

[1] McCollum, Simmonds, Shipley, and Park: Proc. Soc. Exp. Biol. Med., 19, 123, 1921, Jour. Biol. Chem., 51, 41, 1922.

Expose one half of the bone to the rays from a powerful light after immersing it in a 2 per cent. solution of silver nitrate. Wash with distilled water. Examine with a binocular microscope, keeping the bone under water. The calcified area is distinguished by its coating of black metallic silver produced by the action of the light upon the silver phosphate.

As it has been demonstrated that exposure to sunlight or ultra-violet light exerts a powerful curative effect on rickets *animals used for Vitamin D assays must be kept in rooms away from direct light.*

BIOLOGIC ASSAY FOR VITAMIN E

The presence of the anti-sterility factor, vitamin *E* formerly known as Vitamin X may be proven by biologic methods. The method employed for proving its presence or absence in a given product, however, is qualitative only. It cannot readily be adapted to a quantitative measurement of vitamin E owing to the variation in the symptoms of vitamin E deficiency. In the male the germ cell is destroyed whereas in the female in some cases the ovary and ovulation are normal but the developing young die and are reabsorbed. In other cases the deficiency is shown by a lack of milk. If sufficient milk is present the young will show normal growth. If the milk stops, however, the mother will either devour the young or scatter them on the bottom of the cage where they will be found dead.

The following biologic assay method for this vitamin is based upon the work of Sure[1] and Mattill and Clayton.[2]

Apparatus Necessary for Experiment.—Breeding cages equipped with turntable or revolving exercising wheel, food cups, water fountains, scales and weights, chart paper, pipettes, etc.

Animals.—Healthy young albino rats weighing 35 to 45 grams.

Preparation of Experiment.—Put four females and one male rat immediately after weaning in each of four breeding cages of the above description. Place on the following Vitamin E deficient diet:

[1] Sure: Dietary Requirements for Reproduction, Jour. Biol. Chem., 58, 693, 1924; 52, 371, 1925.

[2] Mattill and Clayton: Vitamin E and Reproduction on Synthetic and Milk Diets, Jour. Biol. Chem., 68, 665, 1926.

Casein purified	25 per cent.
Salt mixture (Same as for vitamin D)	4 per cent.
Agar agar	2 per cent.
Cod liver oil	2 per cent.
Dextrin	66.6 per cent.
Yeast vitamin	0.4 per cent.

To the rats in two of the cages feed the product under test in addition to the above diet.

Weigh all rats and plot weight curves every five days. The above synthetic food contains sufficient protein, fat, carbohydrates and mineral salts in the pure form together with adequate quantities of vitamin A and B, to produce normal growth.

Make careful notes as to any destruction of the germ cell in the males and of signs of pregnancy in the females. Remove pregnant females to individual cages three or four days before delivery is expected. In all cases reduce the litter to six in order not to burden the mammary gland and provide an exact method of comparison. The sterility symptoms in the female may manifest themselves in the various ways described above.

If the product under test contains vitamin E in appreciable amount the groups receiving it in addition to the vitamin E free food will show normal reproduction while the control rats will either not reproduce at all or show some of the other sterility symptoms.

BIOLOGIC ASSAY FOR THE P-P FACTOR

Although pellagra-like conditions in dogs[1] and rats[2] have been described there is at the present time no satisfactory method for biologically assaying a product for this substance.

[1] Chittenden and Underhill: Am. Jour. Physiol., 44, 13, 1917.

[2] Goldberger, Wheeler, Lillie and Rogers: Public Health Reports, 41, 297, 1926.

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CHAPTER XIV

TEMPERATURE REGULATION[1]

Constant temperature is one of the most important requirements, necessary to obtain accurate results with the various frog, gold fish and isolated uterus biologic assay methods.

In three published papers I described apparatus for automatically controlling and maintaining the temperatures of water-baths, above or below that of the ordinary room.[2]

Each of the forms of apparatus described in these papers gave very satisfactory results and were used in our laboratory for several years. During the time they were in operation, however, their limitations were studied with the result that details were noted in which improvements could be made. These improvements are incorporated in the apparatus in use in our laboratory at the present time, descriptions of which follows:

Constant Temperature Bath

SPECIALLY DESIGNED FOR FROG AND GOLD FISH EXPERIMENTS

For Maintaining a Constant Temperature below or above That of the Room

There are many forms of thermo-regulators on the market by which the supply of gas or electricity may be automatically controlled in such a way as to maintain constant temperatures in water-baths, hot air ovens, incubators, etc. Most of these, however, are

[1] Abstracted from paper read by the author before the Scientific Section of the American Pharmaceutical Association at St. Louis, Aug. 1927, Jour. A. Ph. A., 10, 1927, 907.

[2] "A Constant Temperature Bath for Maintaining Temperatures Lower than that of the Room," by Paul S. Pittenger, Jour. A. Ph. A. 11, 1916, 1261.

"An Improved Apparatus for Testing the Activity of Drugs on the Isolated Uterus" by Paul S. Pittenger, Jour. A. Ph. A., 6, 1918, 512.

"Temperature Regulator" by Paul S. Pittenger, Jour. A. Ph. A., 5, 1922, 338.

adapted for maintaining temperatures *above* that of the ordinary laboratory or room.

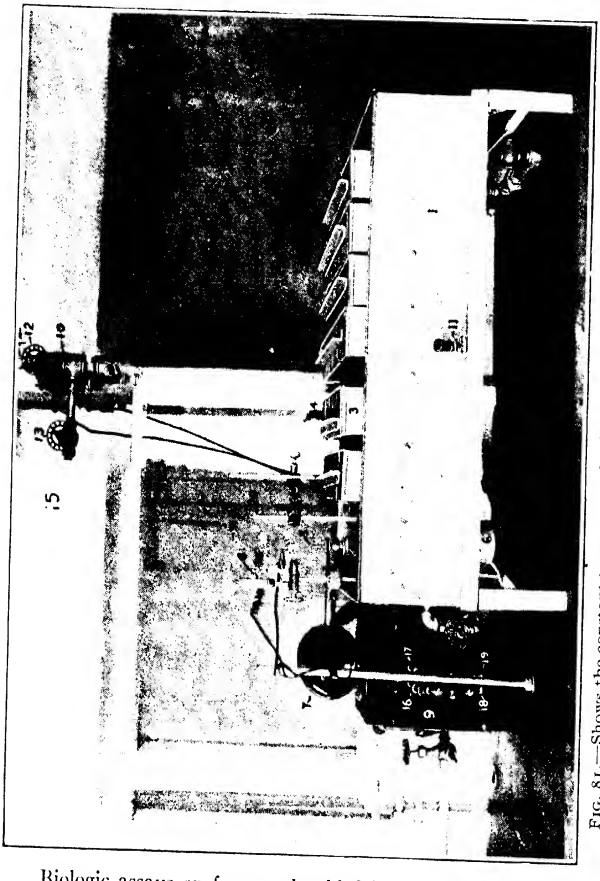

FIG. 81.—Shows the constant temperature bath, with the individual frog cages in place, arranged for maintaining constant temperature below that of the room. (Pharmacologic Research Laboratory, Sharp and Dohme.)

Biologic assays on frogs and gold fish must be carried out at a temperature of 20° C. For example, the "official" description of the assay for digitalis, strophanthus and squills contains the following statement:

"The day before the frogs are to be used, a sufficient number should be taken from the storage tanks and placed in a tank, the temperature of which is approximately 20° C. One hour before the

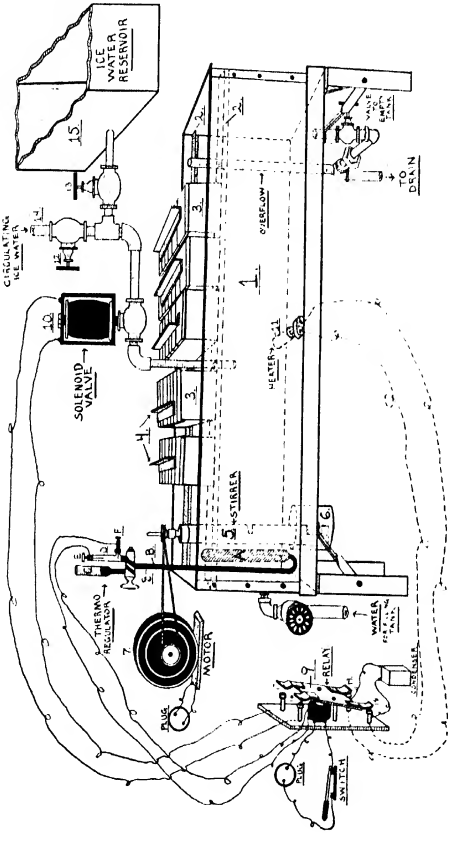

FIG. 82.—Graphic drawing showing the arrangement of the apparatus within the constant temperature bath and method for connecting the thermo-regulator (8 with the relay 9) and the solenoid valve (10). The dotted lines show the method of connecting the relay with the heater (11) when the solenoid valve is disconnected.

assay, they are weighed to within 0.5 gm. and placed in wire cages or containers in a tank containing water to a depth of about 1 cm. (1/2 inch), the water being kept at a uniform temperature of 20° C. during the assay."

As the temperature of many laboratories is above 20° C. during the summer months and below 20° C. during the winter, it is necessary, in order to comply with the official requirements, to have some form of apparatus by which it is possible to automatically keep a water-bath at a constant temperature of 20° C. for 24 hours.

The apparatus described below has been in use in our laboratory for some time and has proven entirely satisfactory.

The complete apparatus is shown in Figure 81. *Figure* 82 is a graphic drawing showing the arrangement of the frog cages, thermo-

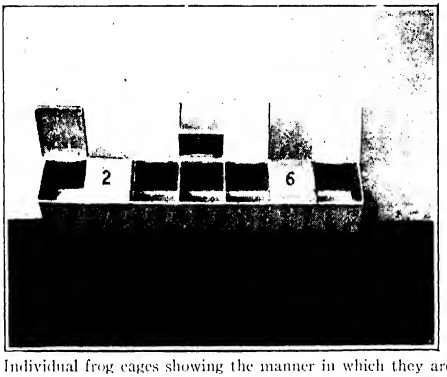

FIG. 83. Individual frog cages showing the manner in which they are divided into seven compartments with hinged lids and wire screen bottoms.

stat, immersion heater and stirrer within the constant temperature bath. Also the arrangement of the solenoid valve, ice water reservoir and method of connecting with circulating ice water system. The labeling of the corresponding parts is the same in both figures and will serve to illustrate the following description;

Briefly summed up this apparatus consists of a metal tank (1) used as a water bath which is provided with two angle-iron supports (2), 1/2″ below the surface of the water, on which the small cages (3) for holding the frogs may be placed.

In the apparatus shown there are six cages of seven compartments each. For details of the construction of these cages see Figure 83. Each of the seven compartments of the cage are approximately 3″

$\times$ 3″ $\times$ 3″. The cages, partitions between the compartments and hinged lids are of galvanized iron while the bottoms of the cages are made of 1 4″ mesh wire screen in order to permit the water to rise to the level of the top of the overflow pipe which is 1 2″ above the angle iron support for the cages. In other words, when the cages are placed on the angle iron support within the tank the water is 1 2″ above the wire screen bottom of the cages. Each cage has seven individual lids the size of the top of each compartment, numbered consecutively. The pieces of angle iron (4) are used to prevent the frogs from opening the lids and escaping.

When the tank is used for gold-fish experiments a perforated metal shelf is placed about 3″ below the surface of the water for supporting the beakers containing the solutions of the drug in which the fish are placed.

In the one corner of the tank is a turbine stirring device (5) dipping into the water, the bottom end of which extends to the bottom of a reservoir (6) which is attached to the bottom of the tank. The turbine stirrer is driven by a motor (7) which lifts the water from the bottom of the reservoir to the top of the tank. This keeps the water within the tank in constant circulation and thus maintains an even temperature throughout the bath.

In the same end of the tank there is a toluol-mercury thermostat (8) which dips into the water and is connected electrically with a relay (9). The relay is so arranged that in the summer it may be connected electrically with a solenoid valve (10) and in the winter with an electric immersion heater (11).

During the summer months when an experiment is to be conducted the toluol-mercury regulator is adjusted to the maximum temperature which we desire to maintain as described later and as soon as the water in the bath rises to this temperature the toluol expands sufficiently to "make" an electrical contact between contact points (16 and 17) which are connected with the coil of the solenoid valve (10) which releases the ice-water, from the ice water reservoir or circulating ice-water system, and thus the temperature of the water in the tank is lowered.

As the contents of the water-bath become cooler, the toluol in the thermostat contracts and the electrical circuit is broken, thereby

automatically shutting off the ice-water supply until the temperature once more rises to the maximum which we desire to maintain.

In our plant we have circulating ice water for drinking purposes which is cooled by an ice machine in the engine room. Through the day while this circulating ice water system is in operation, we utilize it for cooling the tank and thus avoid the necessity of making and storing ice water for this purpose. At night, however, this circulating system is not in operation and it is, therefore, necessary to provide a means of supplying ice water during this time. This is provided for by the insulated ice water reservoir (15). As the U.S.P. method requires that the frogs be maintained at a constant temperature at least twelve hours before the assay, it is necessary that the temperature of the tank be automatically controlled day and night. By opening the valve (12) and closing valve (13) the solenoid valve is connected with the circulating ice water through pipe (14). By closing valve (12) the opening valve (13) the solenoid valve is connected with the insulated ice water reservoir (15).

FIG. 84. A Cutler hammer solenoid valve.

During the winter months the electrical connection between the relay contact points (16 and 17) and the solenoid valve is removed and the relay contact points (18 and 19) are connected with the electric immersion heater (11). After the connections are thus made, as soon as the temperature of the water-bath rises to that temperature at which the regulator was "set" the toluol expands sufficiently to "make" an electrical contact which causes the relay to "break" the current flowing to the electric immersion heater. As the contents of the water-bath become cooler the toluol in the regulator contracts and the electrical circuit to the relay is broken. The relay then automatically "makes" the contact which allows the current to again flow to the immersion heater until the temperature once more rises to the maximum which we desire to maintain.

The construction of the toluol-mercury thermostat is shown in Figure 82. That portion of the glass tube which is lightly shaded represents toluol (*A*) and the black portion (*B*) represents mercury.

In order to adjust the thermostat so that it will throw the relay at 20° C. the two reservoirs *C* and *D* are filled with mercury, the stop-cock placed in the position shown and the bulb (*A*) placed in a water bath. The temperature of the bath is increased until the surface of the mercury just touches the platinum contact wire *E*. The stop-cock is then turned to the opposite position (connecting *C* and *D*) and the temperature of the bath adjusted to exactly 20° C. At exactly 20° C. the stop-cock is again turned to the original position.

The thermostat will then be adjusted so that the mercury will automatically make the connection between the contacts *E* and *F* thus "making" and "breaking" the circuit flowing to the coil of the relay which in turn automatically "makes" and "breaks" the circuit to the heater. Immediately upon completion of an experiment the stop cock of the thermostat should be turned so that the reservoir *C* is connected with *B*. As the temperature of the water bath falls to the room temperature the toluol in bulb *A* contracts and mercury from reservoir *C* passes into *B*. When the apparatus is to be used again the stop cock is left in this position while the temperature of the tank is being raised to 20° C. As the temperature of the water in the bath increases the toluol expands and forces a portion of the mercury back into the reservoir *C*. The temperature should be carefully watched and at exactly 20° C. The stop-cock again reversed so that reservoir *D* is connected with *B*. The thermostat will then automatically maintain the temperature at 20° C.

Before connecting the coil of a relay directly with a 110 volt circuit as described above care should be taken to see that the relay is of the proper type. Many of the stock relays although made to make and break at 110 or 220 volt circuit are equipped with operating coils made to be operated by a 6 volt circuit. A 110 volt circuit would burn out such a relay. With a relay of this type the coil should be connected with a storage battery or transformer.

With a toluol-mercury thermostat as described it is not advisable to pass a 110 volt lighting circuit through the mercury. The relay used in this apparatus, therefore, is a stock Cutler-Hammer Relay #232 (with the contacts normally open) which has the coil so constructed that it is satisfactory for continuous duty and may be connected direct to the 110 volt circuit. Although the voltage remains

the same, the coil reduces the amperage sufficiently to allow the use of the mercury thermostat.

The relay must be of the type shown in Figure 82 which has *four contacts* so arranged that two (16 and 17) will break a contact when the magnet is active.

In the summer months the solenoid valve is connected with contacts 16 and 17 so that when the mercury in the thermostat closes the circuit to the coil of the relay, the relay will "make" the circuit through these two contacts to the solenoid valve, thus releasing the ice water.

During the winter months the wires between the valve and contacts 16 amd 17 are disconnected and wires from contacts 18 and 19 connected. With this "hook-up" when the mercury in the thermostat closes the circuit to the coil of the relay, the relay will "break" the circuit to the heater.

As the heater consumes 500 watts a considerable spark is generated at the make and break of the relay unless a condenser is connected with the two contact points. A condenser of a suitable size connected as shown in Fig. 82 will absorb the spark and prevent the contact points from pitting and sticking.

CONSTANT TEMPERATURE BATH

Specially Designed for Isolated Uterus Experiments

For Maintaining a Constant Temperature above That of the Room

The details of the construction of an apparatus for isolated uterus experiments and a device similar to the above for automatically maintaining a constant temperature bath for Pituitary Assays is described in Chapter VII, p. 99.

CONSTANT TEMPERATURE BATH

Specially Arranged for Maintaining Frogs for Test Purposes[1]

"The proper maintenance of a supply of normal frogs throughout the year, when fresh supplies are not available daily, requires special

[1] Paper read by L. W. Rowe before Scientific Section, A. Ph. A., New York meeting, 1919, Jour A. Ph. A., Nov. 1919, p. 928.

facilities to avoid excessive losses and to insure uniform results when standardizing preparations of heart tonics of the digitalis series by the frog methods.

The chief source of trouble lies in the variation in temperature of the water in which the frogs are stored. In the summer the tap-water in the mains rises to 24° and 27° C., which is too warm, causing epidemics of disease to flourish among the frogs. In the winter the temperature of the tap-water goes as low as 4° C. and at this temperature the frogs are too sluggish. Furthermore, in the winter the sudden change of the frogs from the very cold water to that in which the tests are conducted is not uniformly responded to even if they are placed in the warmer water an hour or two before injections are made.

It was, therefore, thought desirable in our laboratory to control the temperature of the water entering the frog tank so that it would be the same during the entire year. The task becomes more complicated when it is realized that the tap-water must be artificially cooled in the summer and warmed in the winter.

The sketch represents the arrangement of the apparatus and the tank. The tank itself is made of copper and placed in a chamber 4 ft. × 4 ft. × 4 ft., five sides of which are sealed and insulated from room temperature by a thick layer of cork board. The sixth side is a tight fitting refrigerator door with three spaced panes of glass. Since the door is opened only two or three times a day the air in the chamber is maintained at approximately the same temperature as the incoming water. From the sketch it can be seen that three pipe lines, for hot water (*E*), tap-water (*G*), and ice-cooled water (*F*), respectively, supply the tank. The refrigerator for cooling the water is located below the sealed chamber. The thermometer (*A*) shows the temperature of the water entering the tank.

The apparatus which controls the temperature of the water which enters the tank, is known as the Sylphon Tank Regulator and is made by the American Radiator Co. A bulb containing a liquid with a high coefficient of expansion is situated in a metal jacket (*B*) through which the final mixture of warm and cold water must pass. This metal jacket is connected by pressure tubing (*C*) with a needle valve (*D*) in the ice cooled line. The expansion or contraction of the

liquid in the bulb controls the amount of cold water which is admitted by opening or closing the valve. A spring (*H*) connected with the needle valve can be tightened or loosened so that any desired temperature will be necessary to open or close the needle valve.

In summer the hot water is not used at all but enough ice-cooled water is let in by the needle valve to cool a small stream of

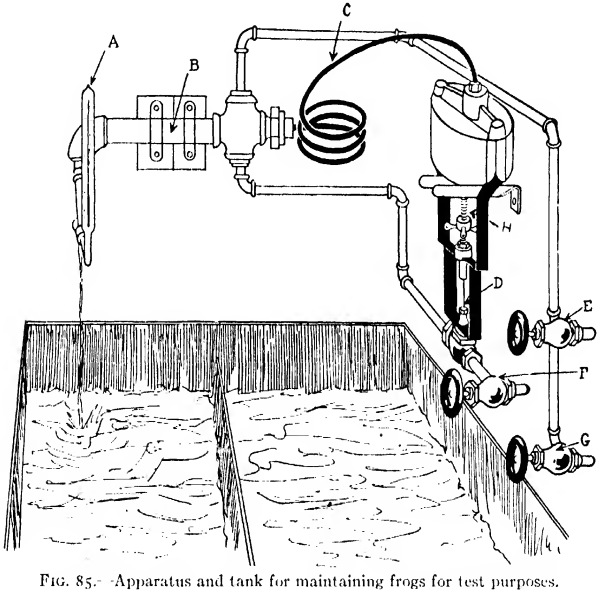

FIG. 85.—Apparatus and tank for maintaining frogs for test purposes.

tap-water to the desired temperature. In winter the temperature of the tap-water is raised to a point a little above that required, by opening the hot water valve slightly, then the needle valve lets in enough water from the cold water line (which in the winter is equivalent to a tap-water line since no ice is placed in the refrigerator) to cool the stream to the desired temperature.

To comply with specifications in the U.S.P., frogs must be kept at 15° C. until wanted for immediate use.

It has been found in this laboratory that this temperature (15° C.) is well suited to the proper storage of normal frogs and with the apparatus described above and the arrangement of it in the sealed chamber we are able to maintain this temperature to within about 1° C. throughout the entire year, if necessary. While very sensitive to improper handling it is equally susceptible to proper adjustment and has been found satisfactory for maintaining an even temperature at all seasons."

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CHAPTER XV

INTERPRETATION OF BIOLOGIC ASSAYS[1]

Most laboratory men know the behavior of drugs which can be chemically tested and can therefore interpret these results and intelligently apply the assay results to the proper adjustment of the products assayed.

Most of these men however have not had sufficient practical experience with Bio-Assays to know the behavior of the various drugs to which the Biologic Assay must be applied.

Therefore, they do not have the necessary experience to properly interpret and apply the results obtained by the assay.

The Biologic Assays themselves can be mastered after limited experience but the proper application and interpretation of the results must be backed by years of experience.

In order to market Biologically Standardized preparations which will satisfactorily meet the new U.S.P. Standards, more is required, therefore, than just a working knowledge of the various Biologic Assay methods.

This knowledge must be baked by a sufficient amount of practical experience to enable one to properly interpret and apply the information gained from the assay in issuing instructions for the proper adjustment of the product assayed.

In other words, one should have sufficient experience to know if a certain preparation tests 200 per cent. whether or not it should be diluted 100 per cent. and whether a reassay is necessary after the dilution. Theoretically it should be diluted 100 per cent. and a reassay should not be necessary. Practical experience, however, has shown that this is not always the case.

[1] "Interpretation of Biologic Assays" by Paul S. Pittenger, Jour. A. Ph. A., 8, 1927, 718.

Whether or not a preparation should be given the full theoretical dilution or concentration as based upon the assay depends upon several factors.

First.—The permanency of the preparation (its rate of deterioration).

Second.—The percentage over or under strength.

Third.—The age of the preparation at the time of the assay.

1. The Permanency of the Preparation

Preparations of Cannabis, for example, are practically permanent and, therefore, are usually as active after one or two years as they were the day tested.

In the case of such preparations it is safe to dilute to the limit and still be assured that the preparations will be of standard strength when they reach the consumer.

On the other hand, such drugs as Digitalis, Ergot[1] and Aconite deteriorate more or less rapidly and dilutions should not be carried too close to the theoretical.

Digitalis.—Some tinctures lose practically no activity within a year, others lose 15 to 30 per cent. within two to three months. In most cases, therefore, preparations of digitalis should not be diluted to less than 110 to 115 per cent. of standard (Higher U.S.P. limit).

Ergot.—A freshly prepared preparation of Ergot in most cases loses from 25 to 30 per cent. within three months. After 4 to 6 months aging the preparation will be found to be almost permanent.

The dilution recommended for Ergot preparations, therefore, should depend largely upon the age of the preparation at the time of assay.

Aconite.—Deterioration is very rapid. Preparations of this drug are sometimes almost inert within a year.

If 2 per cent. Acetic Acid is added to the percolating menstruum the preparation is much more permanent.

Preparations of this drug should not be diluted to less than 110 to 120 per cent. of the average standard (Higher U.S.P. Limit).

[1] A new and reliable Method for the Preservation of Ergot Preparations, by Paul S. Pittenger and C. E. Vanderkleed, Journ. A. Ph. A., August, 1922.

2. The Percentage over or under Strength

If the preparation assays within 100 to 150 per cent. of standard you can usually depend upon the diluted preparation assaying in direct proportion to the dilution. In cases, however, where the strength of the preparation runs as high as 175 to 250 per cent. or in other words where high dilutions are necessary, the diluted preparation will not always test in exact proportion to the dilution.

In these cases it is always advisable to give only about three-quarters the theoretical dilution and re-assay.

This is the safest procedure and usually not more expensive because the increased yield due to the high dilution pays many times over for the re-assay.

It is a good practice in cases where preparations assay 150 to 200 per cent. of standard not to dilute the entire lot at once. This usually produces 50 to 100 per cent. over estimated yield and thus a proportionate overstock which may deteriorate to below standard before being sold. It is best to dilute only sufficient for immediate demand and then reassay the remaining portion and adjust as required.

Deterioration of Dilutions.—High dilutions of some preparations tend to increase their rate of deterioration.

Highly diluted Fluid Extract of Ergot is in this class and a 100 per cent. dilution of a 200 per cent. fluid extract of Ergot is not advisable.

Concentrations.—In the case of under strength preparations the question always arises as to the best method of fortifying. Should the product be concentrated or an overstrength preparation added?

Here again *sufficient experience* is required to judge each individual case. For example, if the case be a S. E. Digitalis as low as 15 or 20 per cent. to be used for manufacturing purposes it is satisfactory to use 5 times the usual formula quantities. On the other hand, if it be an Ergot preparation which had deteriorated to 20 or 30 per cent. it is not advisable to use five times the usual amounts because of the possibility of the presence of toxic decomposition products.

If a preparation is only slightly *understrength* and an over strength preparation is not at hand it is usually advisable to reduce the volume by taking the required amount of the preparation and reduc-

ing it in a high vacuum to an extract. This extract is then dissolved in the remainder of the preparation.

But here again it is necessary to have a knowledge of the behavior of the drug under consideration. Aconite, for example, cannot be treated in this manner because the slight amount of heat, required to concentrate, even in vacuum, splits up the Aconitine into its decomposition products,—Aconine and Benzaconine which are practically physiologically inert. If, however, the *preparation is very markedly understrength,* the above is not a profitable procedure, and would in most cases seriously affect the color and consistency of the preparation.

In these cases it is advisable to prepare a new preparation using an excess of drug in order to obtain an overstrength preparation which can be diluted with the under strength, product.

In some cases this is not practicable as it would produce too large a yield, it is, therefore, advisable to make it a practice not to dump the drug from the percolater until after the assay, as the drug may not have been completely exhausted. Then, if necessary, add additional drug and re-percolate using the under strength preparation as menstruum.

Diluting with Understrength Preparations.—The use of understrength preparations for diluting those that are over strength is usually practicable but there are cases in which it is not good policy.

In the case of *Pituitary Extract,* for example if the preparation is understrength immediately after making it is satisfactory for use in diluting overstrength lots. In some cases, however, you may have a preparation which originally tested 100 per cent. and has deteriorated more rapidly than usual. Experience has shown that it is *not advisable to use such a preparation for dilution purposes,* because such lots have a tendency to start rapid deterioration in the new lot and it is therefore usually more profitable to discard the deteriorated lot.

3. The Age of the Preparation at the Time of Assay

As described in Chapter I, page 8 some preparations deteriorate more rapidly than others and most of the deterioration takes place during the first three or four months.

Therefore, in the case of drugs which deteriorate the *age* of the preparation must be taken into consideration when ordering dilutions. A fresh preparation should not be diluted as close to the theoretical as an aged preparation.

REASSAYS

It is also important to know when reassays are necessary in order to be assured that stocks meet the official requirements.

This depends entirely upon the permanency of the preparation. As before stated, Cannabis for example, is very stable. Therefore, once standardized usually need not be reassayed if properly stored.

On the other hand, stock of preparations of such drugs as Ergot, Aconite and Ditigalis should be assayed at least every three months and readjusted to standard before offering for sale.

In cases where dilutions of 10 to 50 per cent. are made based upon physiologic assay, a reassay of the dilution is usually not necessary. *Reassays, however, are always necessary in the case of high dilutions and after all concentrations.*

Necessity for Supplying Assayist with All Possible Information in Reference to Samples

From this brief description of some of the factors which influence the intelligent interpretation of Biologic assays and their practical application the necessity is apparent for supplying the assayist with all possible information in reference to the product being assayed.

This information should include age, whether a new preparation or a reassay of an old lot, information as to deviations from U.S.P. formulae such as an addition of Acetic Acid to the menstruum in Aconite preparations, etc. In other words, the assayist should have all available information.

Pharmaceutical manufacturers who do not have their own biologic laboratory and must select one to do this class of work for them should carefully consider the following:

1. The experience of the operator in practical biologic assay work.
2. The experience of the operator in the interpretation and practical application of assay results.

It is also important to select a laboratory in which information in reference to products submitted for assay is kept in the strictest confidence.

After selecting such a laboratory or submitting samples to your own analyst confidence should be placed in the laboratory to the extent that all possible information will be furnished in reference to the product submitted.

This is the only way in which the full benefits of Biologic Assays may be obtained.

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CHAPTER XVI

STANDARDS

Although the official and unofficial standards for the different drugs and their preparations are included with the descriptions of the assay methods, they are all included in this chapter with the object of supplying a handy reference containing the standards for all drugs and preparations requiring the biologic test. This should be especially useful to the teacher and routine laboratory worker.

Official Standards

Aconitine, U.S.P., administered subcutaneously to guinea pigs, has a minimum lethal dose of not less than 0.00,000,005,5 gm. and not more than 0.00,000,006,5 gm. for each gram of body weight of guinea pig.

Aconite U.S.P., in the form of the tincture administered subcutaneously to guinea pigs, has a minimum lethal dose, not exceeding 0.0004 c.c. of tincture for each gram of body weight of guinea pig.

Aconite, Tincture, U.S.P., administered subcutaneously to guinea pigs, has a minimum lethal dose of not less than 0.00,035 c.c. and not more than 0.00,045 c.c. for each gram of body weight of guinea pig.

Aconite, Fluidextract, N.F., when assayed biologically the minimum lethal dose should not be greater than 0.00004 c.c. for each gm. of body weight of guinea pig.

Cannabis U.S.P., in the form of the fluidextract, administered by the mouth to dogs in doses not exceeding 0.1 c.c. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by the same dose of the standard fluidextract of cannabis, prepared as directed below.

Standard Fluidextract of Cannabis.—The U.S.P. directs that the standard fluidextract, as supplied by the Bureau of Chemistry, U. S. Department of Agriculture, be prepared as follows:

"Prepare a composite fluidextract, representing at least ten different lots of Cannabis, conforming to the official botanical description, and administer this fluidextract in gelatin capsules to dogs by the mouth. This standard fluidextract must be so adjusted that it will produce incoördination in dogs which have been found to be susceptible to the action of Cannabis when administered in doses of 0.03 c.c. for each kilogram of body weight of dog."

Cannabis, Extract, U.S.P., administered by the mouth to dogs, in doses not exceeding 0.004 gm. for each kilogram of body weight of dog, produces a degree of incoördination equivalent to that caused by 0.03 c.c., for each kilogram of body weight of dog, of the standard fluidextract of cannabis prepared as directed under *Cannabis.*

Cannabis, Fluidextract, U.S.P., administered by the mouth to dogs in doses not exceeding 0.1 c.c. for each kilogram of body weight of dog, produces the same degree of incoördination as that produced by an equivalent dose of the standard fluidextract of cannabis, prepared as directed under *Cannabis.*

Cod Liver Oil, U.S.P.—No oil shall be label as assayed by the U.S.P method unless it contains at least 50 units per gram of oil.

Digitalis, U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 c.c. of the tincture, equivalent to 0.00,000,05 gm. of ouabain, for each gram of body weight of frog.

Digitalis, Tincture, U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 c.c. and not more than 0.0065 c.c. equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each Gm. of body weight of frog.

Digitalis, Fluidextract, N.F., when assayed biologically the minimum lethal dose should be not greater than 0.0006 of the Fluidextract, or the equivalent in Fluidextract of 0.00,000,05 gm. of ouabain, for each gm. of body weight of frog.

Ergot, in the form of the fluidextract, administered by intramuscular injection to single-comb, white Leghorn cocks, in doses not exceeding 0.5 c.c. for each kilogram of body weight of cocks, produces a darkening of the comb, corresponding in intensity to that caused

by the same dose of a standard fluidextract of ergot, prepared as directed below.

Standard Fluid Extract of Ergot. The U.S.P. directs that the standard fluid extract of ergot supplied by the U.S. Department of Agriculture, Bureau of Chemistry should be prepared as follows: Prepare a composite fluidextract, representing at least ten different lots of ergot, conforming to the official botanical description. This standard fluidextract, which must be aged for at least six months before being standardized by the method described in the preceding paragraph, and must be preserved in a vacuum, when administered by intramuscular injection in doses not exceeding 0.5 c.c. per kilogram body weight of cock, produces darkening of the comb of a single-comb, white Leghorn cock which is less than 18 months of age, and which weighs approximately 2 kilograms.

Ergot, Fluidextract U.S.P., administered by intramuscular injection into single-comb, white leghorn cocks, in doses not exceeding 0.5 c.c. for each kilogram of body weight of cock, produces a darkening of the comb, corresponding in intensity to that caused by the same dose of the standard fluidextract of ergot prepared as directed under *Ergota*.

Solution Epinephrine Hydrochloride, U.S.P., diluted with physiological solution of sodium chloride in the proportion of one part of the solution of Epinephrine Hydrochloride to 99 parts of the salt solution, and injected into dogs by the methods described under "Standardization of Epinephrine" (see p. 69), produces a rise in the systolic blood pressure of the dog corresponding to that produced by an equal amount of the standard solution of Epinephrine Hydrochloride prepared as directed below."

"Prepare a standard solution of epinephrine hydrochloride from the standard epinephrine by dissolving 0.050 gm. of epinephrine in 5 c.c. of tenth-normal hydrochloric acid, and dilute this to 50 c.c. by the addition of distilled water, thus making a 1 in 1000 solution. For the assay, add 1 c.c. of this 1 in 1000 solution to 99 c.c. of physiological solution of sodium chloride. This dilute solution (1 in 100,000) must be freshly prepared when needed. On account of the possibility of deterioration, the 1 in 1000 solution must have been recently prepared. It will keep for a short time if preserved in amber-colored bottles in a refrigerator, but it must be discarded if any signs of deterioration, such as discoloration, are observed."

Solution of Pituitary, U.S.P., contains the water-soluble principle or principles from the fresh posterior lobe of the pituitary body of

cattle, 1 c.c. having an activity upon the isolated uterus of the virgin guinea pig, corresponding to not less than 80 per cent. and not more than 120 per cent. of that produced by 0.005 gm. of the Standard Powdered Pituitary, prepared as directed below. The solution should be sterile.

Preparation of Standard Solution.—Carefully weigh a suitable amount of the dried Standard Powdered Pituitary, place this weighed powder in a small agate mortar and moisten with a few drops of distilled water containing 0.25 per cent. of acetic acid. Triturate the moistened powder thoroughly until the whole is of an impalpable frothy consistence. Add a few c.c. of the 0.25 per cent. acetic acid solution and stir the mixture thoroughly. Transfer to a hard glass test tube or breaker; rinse the small agate mortar with the acetic acid solution and add the rinsings to the pituitary mixture; then add enough 0.25 per cent. acetic acid to make the final volume of the mixture of the same number of c.c. as the number of milligrams of dried pituitary powder originally taken. Heat this mixture to the boiling point for not more than one minute and filter. The filtrate contains in each c.c. the active principle of 0.001 gm. of the dried standard powder. Place this solution in hard glass ampuls and sterilize by fractional sterilization for twenty minutes on three successive days at a temperature not exceeding 100° C. Preserve in a cool place (from 5° to 20° C.). This standard solution should not be kept for more than six months.

Standard Powdered Pituitary.—The U.S.P. directs that the Standard Powdered Pituitary supplied by the Bureau of Chemistry, U.S. Department of Agriculture should be prepared as follows: Select a number, not less than 25, of fresh posterior lobes of the pituitary body of cattle, removed within thirty minutes of the death of the animal and carefully free them from all extraneous tissue, immediately after their removal. As soon as they are so prepared, drop them into a flask containing a quantity of acetone corresponding to not less than 4 c.c. of acetone for each pituitary body. Allow them to remain in the acetone for three hours, and then remove them and immediately cut them into small pieces with scissors and place the material in fresh acetone equal in quantity to that first employed. Leave the material in the acetone over night and then remove it and dry it in a vacuum desiccator over calcium chloride at a temperature not above 50° C., for five hours. At the end of this time remove the material and grind it in a mortar until it will pass through a #40 sieve. Dry this powder for at least 12 hours in a vacuum desiccator over calcium chloride at a temperature not over 50° C. Extract this dried powder in a small Soxhlet continuous extraction apparatus with acetone for three hours and again dry in a desiccator over calcium chloride for twelve hours. This dried powder should be preserved in a cool, dark place, in sealed ampuls *in vacuo*, or in vacuum desiccators over calcium chloride until used for the preparation of the standard solution.

Squill U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.006 c.c. of tincture, equivalent to 0.00,000,05 gm. of ouabain for each gm. of body weight of frog.

Squill, Fluidextract, U.S.P., properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.00,055 c.c. and not more than 0.00,065 c.c. of fluidextract, or the equivalent in Fluidextract of not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body weight of frog.

Squill, Tincture, U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.0055 c.c. and not more than 0.0065 c.c., equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body weight of frog.

Strophanthus U.S.P., in the form of the tincture, properly diluted and injected into the ventral lymph sac of a frog, has a minimum systolic dose (the minimum dose producing in one hour a stoppage of the ventricle of the heart in systole) not exceeding 0.00,006 c.c. of the tincture, equivalent to 0.00,000,05 gm. of ouabain, for each gram of body weight of frog.

Strophanthus, Tincture, U.S.P., injected into the ventral lymph sac of a frog, has a minimum systolic dose of not less than 0.00,005,5 c.c. and not more than 0.00,006,5 c.c., equivalent to not less than 0.00,000,046 gm. and not more than 0.00,000,054 gm. of ouabain, for each gram of body weight of frog.

Unofficial Standards

Although the following standards and methods are not official for the various preparations listed, experience has shown that the figures given indicate what may be taken as tentative standards.

Adonidin.

Cat Method.

4.35 mgms. is equivalent to one cat unit.

Apocynum, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.00024 c.c. per gm. body weight of animal.

Cat Method.

0.07 c.c. is equivalent to one cat unit.

Cactus Grandiflorus, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.01 c.c. per gm. body weight of animal.

Convallamarin.

Cat Method.

1.7 mgms. is equivalent to one cat unit.

Convallaria, Fluidextract of Root.

Guinea Pig Method.

M.L.D. of 0.0003 c.c. per gm. body weight of animal.

12 *Hour Frog Method.*

M.L.D. of 0.00025 c.c. per gm. body weight of animal equivalent to 400 H.T.U.

Cat Method.

0.05 c.c. is equivalent to one cat unit.

Convallaria, Fluidextract of Herb.

12 *Hour Frog Method.*

M.L.D. of 0.00015 c.c. per gm. body weight of animal equivalent to 666 H.T.U.

Convallaria, Fluidextract of Flowers.

12 *Hour Frog Method.*

M.L.D. of 0.00009 c.c. per gm. body weight of animal equivalent to 1111 H.T.U.

Digitalein.

Cat Method.

3.5 mgms. is equivalent to one cat unit.

Digitalin (German).

Guinea Pig Method.

M.L.D. of 0.000016 gm. per gm. body weight of animal.

12 *Hour Frog Method.*

M.L.D. of 0.00005 gm. per gm. body weight of animal equivalent to 2000 H.T.U.

Cat Method.

1.5 mgms. digitalin *true* is equivalent to one cat unit.

3.6 mgms. digitalin *German* is equivalent to one cat unit.

0.8 mgms. digitalin Cryst. Nativelle is equivalent to one cat unit.

Digitalis, Tincture.

Guinea Pig Method.

M.L.D. of 0.0025 c.c. per gm. body weight of animal.

12 *Hour Frog Method.*

M.L.D. of 0.015 c.c. per gm. body weight of animal equivalent to 6 H.T.U.

Gold Fish Method.

M.L.D. of 2.85 c.c. in 500 c.c. of water.

Cat Method.

1.0 c.c. is equivalent to approximately one cat unit.

Digitalis, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.00025 c.c. per gm. body weight of animal.

12 *Hour Frog Method.*

M.L.D. of 0.0015 c.c. per gm. body weight of animal equivalent to 66 H.T.U.

Gold Fish Method.

M.L.D. of 0.28 c.c. in 500 c.c. of water.

Cat Method.

0.1 c.c. is equivalent to approximately one cat unit.

Digitalis, Solidextract.

Guinea Pig Method.

M.L.D. of 0.0000625 gm. per gm. body weight of animal.

12 *Hour Frog Method.*

M.L.D. of 0.0005 gm. per gm. body weight of animal, equivalent to 200 H.T.U.

Cat Method.

0.025 gm. is equivalent to approximately one cat unit.

Digitoxin.

Cat Method.

0.3–0.5 mgms. is equivalent to one cat unit.

Ergot, Fluidextract.

Blood Pressure Method.

0.08 c.c. per K. should produce an average rise of blood-pressure of 30 m.m. when injected intravenously into dogs.

Isolated Uterus Method.

Fluidextract Ergot when tested by this method should produce contraction of the isolated uterus of the virgin guinea pig corresponding to that produced by the same dose of the standard fluidextract ergot.

Ephedrine, Sulphate, or Hydrochloride.

Blood Pressure Method.

0.001 gm., intravenously injected, should produce a rise in blood pressure corresponding to that produced by 0.001 gm. of the standard ephedrine sulphate or hydrochloride obtained from authentic Ephedra vulgaris. var. helvetica.

Mydriatic Action.

The unknown solution should produce a threshold reaction in the same strength dilution as that required of the "standard" to produce a threshold reaction.

Euonymus, Tincture.

Cat Method.

4.75 c.c. is equivalent to one cat unit.

Euonymus, Fluidextract.

Cat Method.

0.475 c.c. is equivalent to one cat unit.

Gelsemium, Tincture.

Guinea Pig Method.

M.L.D. of 0.01 c.c. per gm. body weight of animal.

Gelsemium, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.001 c.c. per gm. body weight of animal.

Gelsemium, S. E. or P. E.

Guinea Pig Method.

M.L.D. of 0.00025 gm. per gm. body weight of animal.

Hellborein.

Cat Method.

1.7 mgms. is equivalent to one cat unit.

Hellborus, Fluidextract.

Cat Method.

0.1 c.c. is equivalent to one cat unit.

Jamaica Dogwood, Fluidextract.

Dog.

Should produce incoördination in dogs in doses of 0.55 c.c. per kilo body weight of animal and should not produce incoördination in doses less than 0.5 c.c. per kilo, the drug being administered by mouth after fasting the animal for 12 hours.

Ouabain.

Guinea Pig Method.

The average M.L.D. per gram body weight of guinea pig is 0.000,000, 2 gm.

Cat Method.

0.1 mgm. is equivalent to one cat unit.

Pituitary Anterior, Active Principle, (*Tethelin*).

0.05 gm. per K. dissolved in saline solution and injected intravenously into a rabbit should produce a fall in blood pressure and an increase in the amplitude of the tracing of the heart beat.

Pituitary Extract, (*Anterior*).

Blood-Pressure Method.

0.7 c.c. per K. injected intravenously into a rabbit should produce a fall in blood-pressure and an increase in the amplitude of the tracing of the heart beat.

Pituitary Extract, (*Posterior*).

Blood Pressure Method.

A 1 in 20 dilution of Pituitary Extract intravenously injected into dogs produces a rise in the systolic blood pressure of the dog corresponding to that produced by an equal amount of a 1 in 4 dilution of the standard solution pituitary prepared as described in the U.S.P.

Pituitary, *Posterior Desiccated.*

This powder should possess 50 per cent. of the activity of the "standard pituitary powder."

Scillitoxin.

Cat Method.

0.4 mgm. is equivalent to one cat unit.

Squills, Tincture.

Guinea Pig Method.

M.L.D. of 0.0025 c.c. per gm. body weight of animal.

Cat Method.

5.75 c.c. is equivalent to one cat unit.

Squills, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.00025 c.c. per gm. body weight of animal.

12 Hour Frog Method.

M.L.D. of 0.0012 c.c. per gm. body weight of animal equivalent to 83 H.T.U.

Cat Method.

0.575 c.c. is equivalent to one cat unit.

Strophanthus, Tincture.

Guinea Pig Method.

M.L.D. of 0.0001 c.c. per gm. body weight of animal.

12 Hour Frog Method.

M.L.D. of 0.000075 c.c. per gm. body weight of animal equivalent to 1300 H.T.U.

Cat Method.

0.03 c.c. is equivalent to one cat unit. (Strophanthus Kombe.)

0.015 c.c. is equivalent to one cat unit. (Strophanthus hispidus.)

Strophanthus, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.00001 c.c. per gm. body weight of animal.

Cat Method.

0.003 c.c. is equivalent to one cat unit. (Strophanthus Kombe.)

0.0015 c.c. is equivalent to one cat unit. (Strophanthus hispidus.)

Strophanthin.

Guinea Pig Method.

M.L.D. of 0.000001 gm. per gm. body weight of animal.

Strophanthin Amorph.

Cat Method.

0.13 0.17 mgms. is equivalent to one cat unit.

Veratrum, Tincture.

Guinea Pig Method.

M.L.D. of 0.002 c.c. per gm. body weight of animal.

Veratrum, Fluidextract.

Guinea Pig Method.

M.L.D. of 0.0002 c.c. per gm. body weight of animal.

Veratrum, S. E. or P. E.

Guinea Pig Method.

M.L.D. of 0.00005 gm. per gm. body weight of animal.

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CHAPTER XVII

CARE OF ANIMALS[1]

The accuracy of the biologic test to a marked degree depends upon maintaining the health and vigor of the test animal.

In most laboratories all of the test animals are of necessity kept indoors. If animals are to be kept in a healthy condition indoors for a prolonged period, special care must be exercised in the construction of cages, feeding and the elimination of vermin.

If cages are improperly constructed they cannot be readily cleaned and become offensive and vermin ladened. All cages should, therefore, be so constructed that they may be readily cleaned, disinfected and sterilized. Animal rooms containing properly constructed cages can be easily cleaned and kept free from vermin.

When kept indoors some animals require special additions to their food.

White Leghorn Roosters for testing ergot, for example, are normally outdoor animals. When placed in indoor laboratory cages for a period of one year they require more than a mixed grain scratch feed. To be kept in a healthy condition, in addition to mixed scratch feed, they require growing mash, grit and charcoal.

Owing to the fact that they do not have the hard ground and gravel in which to scratch and pick for food, the nails and beak if not properly cared for will become abnormally long. The nails become so long and curved that it is difficult for the cockerel to walk or hold fast to the roost. The beak will grow until it is impossible for the cockerel to close its mouth or to eat. It is, therefore necessary at regular intervals to clip the beak and nails with bone clippers.

[1] Paper by the author read before the Scientific Section of the American Pharmaceutical Assn. St. Louis, Aug., 1927. Jour. Amer. Pharm. Assn., March, 1928.

Animals which are allowed but little exercise require less and oftimes different food than those that receive abundant exercise. The dog, for example, is a carnivorous animal, and meat is his natural diet. When kept indoors, however, and allowed but little exercise, the cooling effect of vegetables is necessary to offset the overstimulating effect of excessive meat eating.

It is not the object of this chapter to treat in detail the methods of housing, breeding, raising and care of each individual laboratory animal, as books upon these subjects are available. Practically all of these books, however, consider the animal only in its natural environment. The author on the other hand has had considerable experience with the housing and care of laboratory animals. The object of this chapter, therefore, is to describe simply and briefly the cages and methods which we employ at the present time for housing laboratory animals and keeping them in a healthy condition.

General Principles

The animal house or room should be so arranged that it will possess maximum comforts for the animals and conveniences for the attendant. It should be dry, cheerful, well ventilated, warm in winter, cool in summer and easy to clean and keep in a sanitary condition. The house or room should have plenty of ventilation but *care should be exercised to avoid drafts.*

If possible arrange room so it will admit plenty of sunshine.

All cages should be made of metal in order that they may be easily washed, disinfected and sterilized. Cages for all kinds of laboratory animals should be equipped with removable metal trays. Sawdust should be placed in the trays to absorb all drippings. The trays should be emptied, washed and disinfected daily and sterilized at least monthly.

The sawdust removed from trays should be emptied into large galvanized iron ash cans *with lids.* Cans should be emptied and disinfected at regular intervals.

The room should be provided with a large metal sink in which the trays may be washed and scalded. Sink should be equipped with hot water and if possible live steam. After washing and scalding the

trays a small quantity of a non-poisonous disinfectant should be spread over the bottom and clean sawdust placed on top. The trays are then replaced in the cages. In the case of trays for guinea pig or rabbit cages a bedding of hay should be placed on top of the sawdust.

If this procedure is followed a large number of animals may be kept in the laboratory without objectional odors or danger of vermin.

The cages should be "washed down" occasionally with an antiseptic solution and if live steam is available they should be sprayed with it at regular intervals in order to kill vermin which may be in the crevices, etc.

The animal room can be kept free from roaches, etc., by dusting a mixture of Sodium Fluoride 50 per cent. and Sodium Borate 50 per cent. into all cracks, along the bottom of all partitions, under cages, etc.

All food outside of the cages should be kept in closed metal containers in order that it will not attract rats, mice and other pests.

If the animal house is on the first floor without a basement underneath, the floor should, if possible, be made of asphalt or cement. If a board floor is used it should be built far enough above the ground and with openings large enough to permit small dogs and cats to go under after rats.

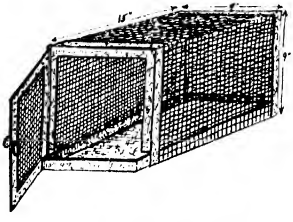
CAGES

Practically all cages should be of all-metal construction. It is more economical, however, in the case of extra large cages to build the framework of wood and make the trays, partitions, doors, etc., of metal. Several coats of aluminum paint should be applied to the wooden parts of such cages to prevent the absorption of moisture. All cages may be more or less of the same general design. Four different size cages usually suffice for the animals required for biologic assays. These may be divided roughly into small, medium large and extra large.

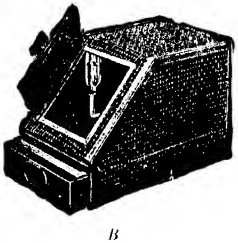
The author usually designs and builds special cages to suit the particular room in which they are to be used. The stock cages, however, shown in Figs. 86, 87, 88, 89 and 90, built by Geo. H. Wahmann

Manufacturing Co., 520 W. Baltimore St. Baltimore, Md., are very satisfactory for practically all purposes.

Small Size Cages. The small cages shown in Fig. 86 are of suitable size for housing four or five guinea pigs or rats. This size cage is also suitable for holding one rabbit or one small cat while under test.



A



B

FIG. 86*A*.—Small size cages. Dimensions 9″ × 15″ × 9″ high. Made of heavy wire mesh 3 holes to an inch. No. 18 wire. The pan inside is heavy galvanized iron. The cage may also be equipped with a holder for water bottle the same as shown in *B*.

FIG. 86*B*.—Dimensions 9″ × 9″ × 15″ deep. Made of galvanized American ingot iron, and galvanized wire cloth. Galvanized iron drawer is removable for cleaning. Provided with water drinking fountain and holder.

Medium Size Cages.—The cages shown in Figs. 87 and 88 are of a very serviceable size. Each unit is large enough to house 25 guinea pigs or rats, one small dog, five small or three large rabbits, three small or two large cats. This size cage is also very satisfactory for holding one or two roosters during the test period. They are, however, too small for housing roosters or dogs for longer than the

test period. The *feed boxes* shown in Fig. 88(*B*) are specially designed for feeding roosters. They can be conveniently hung inside of cages shown in Figs. 87, 88, 89, 90 and 91. They are divided into three compartments for holding charcoal, grit and growing mash.

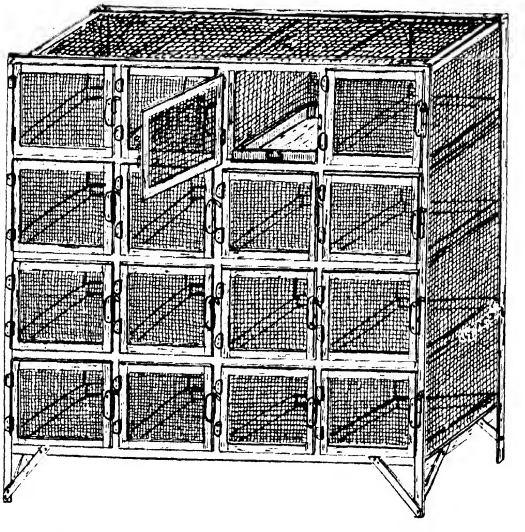
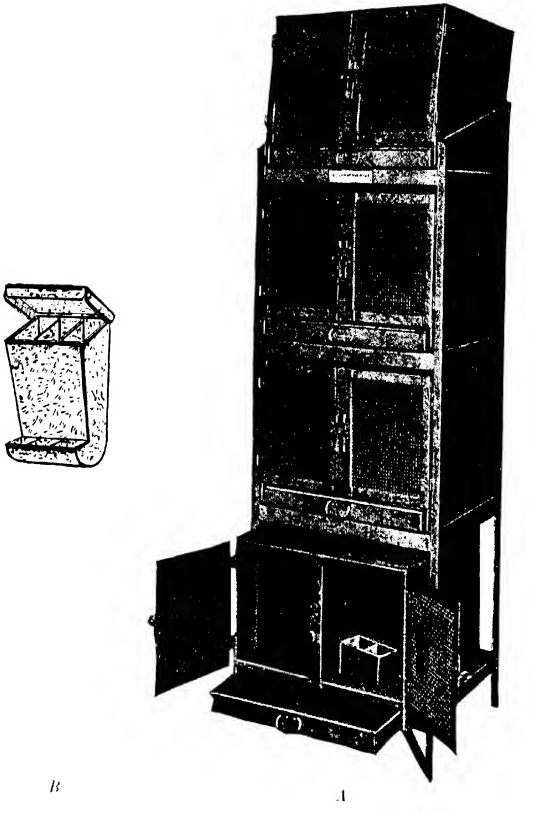


FIG. 87.—Medium size cages. Each unit of this cage measures 22" × 20" × 14" high. Made of heavy galvanized iron and stout wire mesh on a frame of angle steel. A cage of this type may be built so that it consists of 1, 8, 12 or 16 units. The galvanized iron trays are removable for cleaning.

Large Size Cages.—Cages of the size shown in Figs. 89 and 90 are satisfactory for housing one large or two small dogs, three or four large cats, five or six rabbits or two roosters. The cage shown in Fig. 90 is constructed with a removable wire floor and tapered bottom for collection of urine for metabolism experiments. Solid galvanized iron extends to one half the height of the sides and door inside to insure the collection of all urine voided. This also prevents dirt



B *A*

FIG. 88. Medium size cage. This cage (*A*) is of a slightly different construction than that shown in Fig. 87. This type cage was originally constructed for housing cats, but its peculiar construction makes it satisfactory for all small animals, even small dogs. This cage may be made with as many units as desired. Each unit is 22″ wide, 20″ deep and 15″ high. The four-units of the cage shown above are supported by a stand made of heavy angle iron. A solid galvanized iron shelf is placed between each cage to prevent dirt from the cage above dropping into the cage underneath. *B* shows feed box for roosters. May be placed inside of cages, 87, 88, 89, 90 or 91.

from the upper cage from falling into the lower cage. Two hooks are soldered to the bottom, one on either side of the outlet, for attaching the specimen collecting bottle.

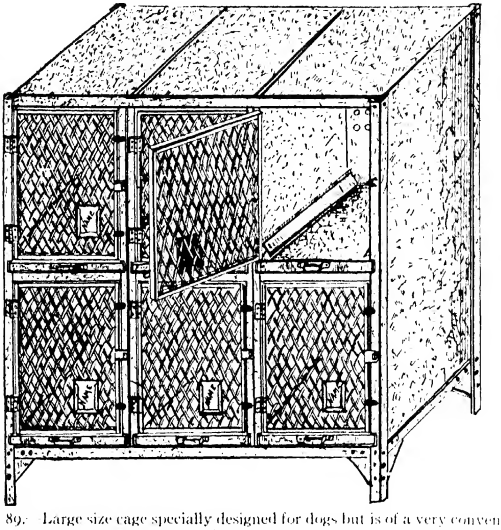


FIG. 89. Large size cage specially designed for dogs but is of a very convenient size for cats, rabbits or roosters. Each unit measures 32″ × 34″ high × 31″ deep. The cage is made with solid sides and back, two cages high. Can be made as many cages to the row as desired. Made of heavy galvanized iron throughout. The door is made of galvanized angle iron with heavy galvanized diamond mesh.

Extra Large Cages.—The two story cage shown in Fig. 91 was designed by the author for housing roosters, dogs, cats, rabbits or guinea pigs. Each unit of the cage measures 48″ long × 30″ wide × 30″ high. The framework is made of 2″ × 3″ white pine planed on all four sides. The sides, doors and fronts are made of perforated black iron. The door frames and supports are of 1″ angle iron. The trays are made of heavy galvanized iron supported by three pieces of 1″ angle iron. The angle irons are the only means of sup-

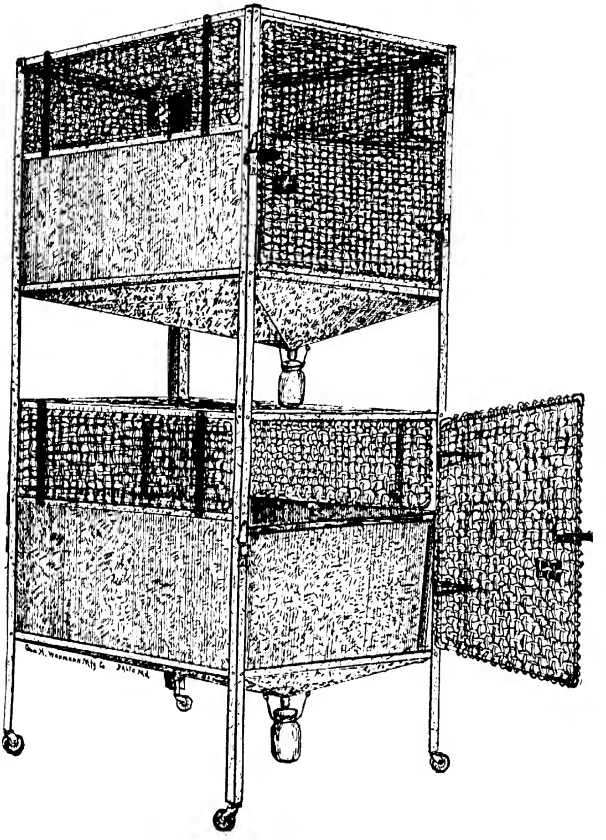


FIG. 90.—Large size cage. The inside measurements of each cage are 22″ wide, 32″ deep, 22″ high. The framework is of heavy angle iron. The door and sides are constructed of very heavy special steel wire mesh made over a framework of pipe. The bottom, slanting pans and sides are of heavy galvanized iron. The framework is of one piece from bottom to top to insure rigid construction.

port for the trays. When the trays are removed, therefore, all dirt may be swept through to the floor. The cages may thus be easily and thoroughly cleaned. The twelve units of the cage are separated from each other by means of sliding removable partitions A made of perforated black iron in a frame of heavy galvanized iron. All metal parts are painted with aluminum paint to prevent rusting. The removable partitions make it possible to divide the cage into

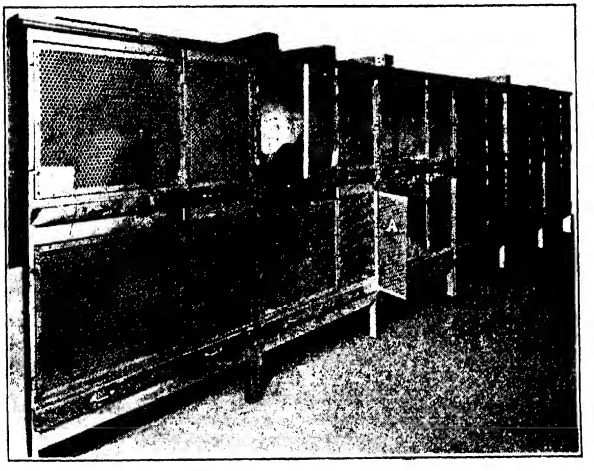


FIG. 91.—Extra large cages. Designed by the author for housing roosters, dogs, cats, rabbits or guinea pigs. The removable partitions make it possible to divide cage into any desired number of units from two to twelve. Each unit of the cage measures 48″ long × 30″ wide × 30″ high. The galvanized iron forms B are used to cover the space between two trays when a partition is removed.

any desired number of units from two to twelve. In other words it may be made into two very large cages, four extra large cages or six, eight or twelve large cages, depending upon the number and kind of animals to be housed. It is better to house six to eight roosters or three or four dogs in two units (without partition between) of a cage of this size than to house one or two animals in smaller cages. By using two units as one cage the animals have plenty of room for

exercise and are, therefore, healthier than when each animal is placed in a smaller cage.

The removable partitions are especially convenient for ergot and cannabis assays for isolating animals during the fasting and testing period. At the beginning of the fasting period all of the roosters may be driven into one of the two units and the partition inserted. The other tray is then emptied and supplied with fresh sawdust. The cocks to be fasted are placed in this unit. This procedure precludes the possibility of obtaining scratch feed, from previous feedings, which may be mixed with the sawdust. All mash hampers must of course be removed or covered. During cannabis assays the perforated metal partitions are replaced by solid partitions so the animals cannot see each other.

CATS

The cat is a mammal belonging to the order carnivera and, therefore, prefer to go in quest of their own prey and kill it.

All cats have the same general type of structure, the variations being only in minor characteristics which are very slightly related to their habits.

Cats are found all over the world except in Australian region, Madagascar and the West Indies. They are tropical and heat loving; a few specimens, however, are found in the far North. For example, the tiger in Asia and the Puma in America.

Varieties. The domestication of the cat occurred at a very early period. The first indication of its connection with man is found in the ancient Monuments of Egypt, Babylon and Ninavah.

The *long-haired cats* include the various families known as Indian, Chinease, Angora and the Persian.

The *short-haired or western cats* include several varieties which are based mostly upon color.

The common varieties include the tortoise shell, tortoise shell and white and the Tabbies. The tabbies are divided into four classes: The brown, spotted, blue or silver and the red.

Short-haired cats are preferable for laboratory use.

Housing.— The cat as a rule is a very clean animal and when kept in the laboratory should be provided with means for remaining so.

The sawdust in the trays of the cat cages should be changed daily. Figures 87 and 88 show cages suitable for housing cats.

Exercise.— Animals which are kept for a prolonged period should be provided with some means for exercise. It is good practice to keep two units of a cage of the type shown in Fig. 91 for use as an exercising cage. Several animals should be removed at a time from the small individual cages and placed in the large cage for twelve to twenty-four hours.

Feeding.—The cat thrives best on a mixed diet of milk, meat, fish and vegetables. If fed too much meat or fish it will refuse vegetables. It is best, therefore, to finely grind the meat or fish and vegetables and thoroughly mix so it will be impossible for the animal to separate the meat or fish from the vegetables. The meat may be either raw or cooked. Animals with a limited amount of exercise, however, should not be given an abundance of raw meat. When fresh fish is not available, canned salmon may be used to advantage. Oatmeal porridge serves as a variation which is acceptable to most cats.

Water.— Water should be supplied in vessels that may be quickly and thoroughly cleansed. Do not allow drinking water to remain day after day without change. Frequent change of water should be provided and placed where the sun will not shine upon it.

Fleas.—As a precaution against fleas the cages should occasionally be washed down with a solution of Sanax and steam sterilized. The tray supports should be dusted with a mixture of Sodium Borate and Sodium Fluoride. If the animals become infested with fleas, spray the cages and room with kerosene and wash the animals in a weak solution of Sanax. Care should be taken to thoroughly wash out the antiseptic solution with fresh water to prevent possible irritation of the skin.

Worms.—Worms are very often found in both the stomach and intestines of laboratory cats. The usual symptoms are those of intestinal irritation and the unthrifty appearance of the animal. The fur becomes harsh and dry and the abdomen distended.

The animal should be fasted for 12 hours and then given by mouth from 1/10 to 1/4 grain of Santonin with 1/4 to 1 grain of Calomel depending upon the size and age of the animal. This should be

followed after four to five hours with a dose of 1/2 *to* 1 ounce of mineral oil administered by stomach tube.

Mange.- Two forms of this disease attack the cat, sarcoptic and follicular. Both are produced by a parasite which multiplies rapidly.

The ordinary or sarcoptic mange is due to a small parasite which burrows under the skin, where it deposits its eggs, which upon hatching become very irritating to the skin, as the young insects grow and tunnel out to the surface, where they breed for the next generation to burrow and deposit its eggs.

The sarcoptic mange may appear first on the face and cheek, or upon the insides of thighs and armpits, and may extend over the whole body. On account of the intense itching the animal will scratch itself, which produces abrasion, which scabs over and when scratched off becomes sores.

In follicular mange the parasite affects the nose around the muzzle, the skin of the chin and the skin of the paws, surrounding the claws, where it burrows in beside the roots of the whiskers and larger and grosser hairs, and into the sebacious glands. On examination around the roots of the whiskers and on the skin of the paws, we find dark red pimples or pustules about the size of a pin head.

A cat with mange should be separated from the other animals, washed with antiseptic soap or a weak solution of Sanax or other antiseptic solution and one of the standard mange remedies applied.

Other common diseases of the cat include catarrh, distemper, pleurisy, pneumonia, bronchitis, constipation and diarrhoea.

Constant attention should be devoted to the prevention rather than the cure of these diseases.

DOGS

In the case of dogs for laboratory use, attention should be paid primarily to cleanliness, feeding, exercise and affection.

Kindness runs strong in a dog's nature. All domestic animals are sensitive to the kindness of their owners and attendants, but no animal is so dependent upon human sympathy as the dog. Laboratory dogs may, therefore, be handled more easily by an attendant

who is fond of and kind to the animals than by one who simply cares for them as part of his necessary daily tasks.

Varieties.—For *blood pressure work* any type or breed of dog may be satisfactorily employed. Usually, however, a common mongrel, short-haired, medium sized dog is to be preferred.

For *testing Cannabis* medium sized fox terriers are the best. If these are not available, any medium sized, short haired, long legged dogs are preferable to long-haired, short legged dogs.

Housing.—Dogs which are kept indoors should be placed in cages of the design shown in Figs. 90 and 91. Trays should be filled with sawdust and changed daily. (See page 235.) If the animals are kept outdoors they should be provided with kennels. The kennels should be protected from rain and cold and should be ventilated from the top in order to prevent drafts. The animals should not be allowed to sleep on cement floors as cement is often cold and damp and will cause rheumatism and lameness. If the floor is made of cement a bed of straw should be provided and frequently changed. The kennel entrance should be from the side rather than from the front to safeguard against rain and wind. If possible shade should be provided in summer.

Exercise.—Small and medium sized dogs such as used for laboratory purposes require plenty of exercise. If the dogs are not given sufficient exercise they overeat, become sluggish and ofttimes sickly.

If the size of the animal house permits three or four dogs should be housed together in two to four units of a cage of the type shown in Fig. 91 rather than kept in individual cages of the type shown on page 235.

In the large cage the dogs play and run and romp together thus getting plenty of exercise. If lack of space makes it necessary to house each dog separately, one large exercising cage should be provided in order that the animals may be exercised in groups.

Feeding.—Dogs especially when kept indoors are better off a little hungry rather than overfed.

Feeding but once a day is the custom at most large kennels, but I consider twice a day much preferable. Twenty-four hours is too long a period for the stomach to go without food, and the animal

so fed will usually bolt the food without proper mastication. This is one of the chief causes of indigestion.

Dogs should not be fed continually upon the same diet of cooked or raw meat, starchy food or corn bread. Dogs thus fed suffer from fermentative indigestion. No one food contains the necessary protein, fats and carbohydrates. A single meat diet creates a distaste for other and more varied foods. Do not feed such meats as pork, veal, etc. Feed beef, mutton and, occasionally liver. Do not feed lights or offal from slaughtering.

If the dogs are fed twice a day dog biscuits are an ideal food for *the morning meal.* They should be fed dry. For small dogs the biscuits should be broken into small pieces. Dog biscuits contain beef, cereals, ground bone and flour and, therefore, furnish a well balanced diet. The hard biscuits provide exercise for the teeth and prevent bolting.

The evening meal should vary otherwise it will become distasteful. Boiled vegetables, with meat should be fed during the summer months. Vegetables of all kinds except potatoes and corn (which are too difficult for a dog to digest) should be mixed with the boiled meat.

Soups that are not too greasy make excellent food and add variety to the diet. Dry broken bread or broken dog biscuits added to soupy food also adds variety.

In our laboratory we prepare the evening meal fresh daily. Small pieces of beef necks, flanks or butchers scraps and bones are placed in a large pot and thoroughly boiled with plenty of water and a little salt. The bones are then taken out and a quantity of vegetables added and boiled to shreads. For variety the vegetables are replaced with rice, broken bread or dog biscuits.

Each dog is given some of the thoroughly cooked bone. This induces the dog to exercise his teeth and salivary glands while the small particles of bone gnawed off constitute nutriment to the bony structure.

Care should be exercised in feeding of bones. Many dogs are injured by careless feeding of bones with sharp points or pieces which break off and are swallowed. Chipped bones are always dangerous. Small bones, such as chicken bones, splinter very

easily and should never be fed as they are liable to cause trouble either by lacerating or puncturing the stomach or intestines.

Never let food stand around after meals. After the dog is through with its regular meals, remove all food, otherwise he will eat too often and too much.

Water.—Same as for cats. (See page 239.) For dogs housed outdoors a pan placed under a dripping spigot makes an ideal dog fountain.

Fleas.—Same as for cats. (See page 239.)

Worms.—If a dog has a ravenous appetite, does not seem to thrive and has a bloated appearance of the abdomen while thin elsewhere treat it for worms in the same manner as described for cats. (See page 239.)

Mange.—Same as for cats. (See page 240.)

Lice.—Lice cause great irritation which causes the animal to scratch and worry constantly. They live on all or any part of the body, but are usually most abundant about the head and face, the eyes, roots of ears and along the top of the back where it is hard for the dog to get at them. By closely examining the animals skin, red streaks and spots of blood will be seen where the louse has been feeding.

A mixture of 40 per cent. Sodium Fluoride and 60 per cent. Talcum dusted upon the skin will kill the lice. Lice may also be killed by washing the animal in a solution of Sanax or some similar antiseptic. Care should be taken to prevent the solution from coming into contact with the animals' eyes.

If the cages and animal quarters are kept clean and are frequently sterilized the animals will be free from lice unless the lice are brought into the cages by "new animals."

Nails.—The lack of hard ground and gravel in which the animal may scratch results in an abnormal growth of the nails. This makes it difficult for the animal to walk. The nails should, therefore, be clipped occasionally with bone clippers. Care should be taken not to clip the nail too close to the toe or this will cause bleeding. By careful observation the extent to which the blood enters the nail can be seen and by carefully avoiding the cutting of this part bleeding will not occur.

FROGS[1]

Distinction of Batrachians from Fishes and Reptiles.—"The Batrachians represent a Class of Vertebrate animals occupying a position between Fishes and Reptiles. There is considerable variation in general appearance among the different living members of the Class, so that a Batrachian is not as easily defined and identified as is a fish, a bird, or a mammal. There is no one characteristic by which it may be known, as there is in each of these other Classes."

Two Orders of Living North American Batrachians—Urodela and Salientia.—"The living North American Batrachians differ enough to allow classification into two distinct Orders, the Urodela and the Salientia. The Urodela are the Tailed Batrachians, or Salamanders, with various popular names, such as Mud Puppies or Water Dogs, Tritons, Newts, and Efts. The Salientia are the Tailless Batrachians, *i.e.*, the Toads, Tree Frogs, Frogs, and all Batrachians that have the frog-like form."

Development and Metamorphosis.—"The North American representatives of the Salientia deposit the eggs in water, usually in shallow, stagnant water. The eggs may be laid singly or in small clusters, as in the case of some of the tree frogs; in large masses, as is the habit among the frogs; or in long unbroken strings, as in the case of toads. The eggs are sometimes free in the water, but more often are attached to water-weeds or other objects. If free, the egg mass has such buoyancy that it floats at the surface of the water (Rana sylvatica)."

"The egg is spherical, and is provided with a large amount of light-colored yolk. When the eggs are first laid, part of this yolk can be seen occupying the lower portion of the egg under the more or less pigmented upper portion. The dark pigmented portion of the egg at the top (later the whole surface becomes black or brown in colour) allows a greater absorption of the sun's heat than would a lighter colour."

The time required for the development of the frog's egg varies with the species and with the temperature in case of a given species. The eggs of the Rana pipiens hatch in two to three weeks.

[1] Quotations from "The Frog Book" by Mary C. Dickerson.

"The length of life of the tadpole before its change into the frog may vary from two or three weeks to as many years. Some of the interesting points possible to observe during the metamorphosis are the following: The arms and legs develop simultaneously, but the arms are concealed under the opercular membrane. The horny parts of the mouth are dropped and the mouth cavity increases in size. The tail becomes smaller by absorption from within. The tadpole takes on habits of rushing to the surface or of resting wholly out of the water, showing that the lungs, which for some time before had been functional in company with the gills, now take on all of the respiratory work (except that performed by the skin)."

The Families of the Salientia or Tailless Batrachia.- "The Tailless Batrachia of North America, as far as known, are represented by seven families, containing together twelve genera and fifty-six species."

Frogs belong to the family Ranidae.

"The family contains a large number of genera, and it is very widely distributed. Australia, New Zealand, and southern South America are the only parts of the world not possessing representatives of the true frogs. Some two hundred species (by far the largest number) are found in the tropical portion of the Eastern Hemisphere. North America has only one genus, Rana; there are seventeen known species, the one form of the rank of a sub-species (Rana c. latiremis). They are most numerous in the eastern part of the continent, though several species are found west of the Sierra Nevada Mountains."

The official species, used for the assay of the cardiac stimulants, is *Rana pipiens* Shreber, commonly known as the Leopard Frog, Spring Frog and Grass Frog.

The color of the official leopard frog is green, grey or brown, with somewhat rapid change from one color to the other.

"The Leopard Frog is one of the most beautiful in colouring of all our common frogs. It is better known than others, not only because of its wider distribution and greater numbers, but because it has the habit of going considerable distances from its pond, or marsh."

Frogs of about 2 1/2 inches in length are the proper size for assay purposes.

Age of Leopard Frogs.– The age of a leopard frog may be approximately estimated from its body-length. The average body-length of the official leopard frog is as follows: One year old, one and one-half inches; two years old, two inches; three years old, two and one-third inches and four years old, two and three-quarters inches.

Sex.– The sex of the frog may be distinguished as follows: The *male* leopard frog has the thumb of the fore foot much enlarged on the inner edge and has a vocal sac between each ear and shoulder. These vocal sacs can be demonstrated by seizing the frog around the waist just in front of the hind limbs and alternately squeezing and relaxing the pressure. In this way the male will inflate the sacs.

The ripe *females* are very gravid and swollen and have no vocal sacs and no enlarged thumbs.

When disturbed frogs jump for safety. The jump is oftimes accompanied by the squirting of water. This habit of squirting water– as though in the face of an enemy– may sometimes result in protection to the frog, since the water is disagreeable in odor.

When the frogs are removed from the tank for assay purposes each individual animal should be held until it has finished squirting water before being weighed.

The slippery skin, the slender body and the strong hind legs make it difficult to hold a frog. He gives most unexpected and vigorous jerks of the body to free himself. They are most easily held by firmly gripping about the posterior part of the body and thighs.

Temperature and Hibernation.–"All Batrachians, like Fishes and Reptiles, are cold-blooded, *i.e.*, they have a variable body heat, depending on the temperature of the environment. In this they are distinguished from Birds and Mammals, which have an unvarying body temperature.

"Because of this variation of the body temperature with that of the surroundings, Batrachians can endure extremes of heat and cold, but are greatly influenced by them. With decreasing temperature the processes of respiration and circulation gradually slacken speed, and the animals become more and more lethargic until they sleep. With rise of temperature to a limit varying from 20° to 30° Centigrade in the different species, they gain increased activity."

This variation in activity which accompanies variations in temperature also produces variations in the susceptibility of the frog to the drug under test. This is the reason that the U.S.P. requires that frogs used for assay purposes must be kept at a temperature approximately 20° C. for 24 hours before the test and at exactly 20° C. for one hour previous to and during the test period.

Shipping. There are several methods of shipping frogs. Some dealers ship in wet boxes without moss or hay. Others ship in wet moss. The author has found, however, that a large percentage of the frogs die during shipment if not properly crated. The method of packing should be governed by the temperature at time of shipment. *In the very hot summer months* they should be placed in boxes with wet moss. The one side of the box should be covered with fly screening. Care should be taken not to use too much moss as this mats and kills the frogs. The greatest loss of life occurs when too many frogs are placed in a single box or compartment. In such cases the frogs and moss mat in one corner of the box which kills a large number of the animals. Not more than 30 frogs should be shipped in one box or compartment. On large shipments the boxes should be divided into compartments holding 30 to 35 frogs each, *loosely packed* with wet moss. *During the winter months* frogs may be packed as described above for shipping short distances. For shipping long distances they should be packed in loose moist (not wet) soil covered with about four inches of hay. *The hay must not be wet.* Do not ship more than 40 or 50 frogs in one box or compartment.

"Members of the Salientia can endure an astonishing amount of cold; even freezing in the water of a pond or in the mud at its bottom will not of necessity cause death. The circulation and all life processes may stop, but if the blood and protoplasm of the heart do not fall much below freezing-point, the frozen parts will recover. Since sleep is induced in specimens at varying temperatures below 10° C., it is easy to understand why Batrachians are not found in the extreme north."

"This hibernation or sleep induced by cold continues until a return of high temperature."

Frogs that become frozen during shipment may, in many cases, be revived and used for assay purposes. Frozen frogs, however,

should not be placed in a tank of water to "thawout" as this rapid thawing usually kills most of them. Frozen frogs should be placed in a cold place where they will thaw out gradually. If this procedure is followed very few of the frogs will die unless they have been frozen for too long a period of time.

Food.—"The frog's method of eating is attended with much nervous alertness, and sometimes with unsatisfactory results to the frog. The food consists, in general, of living worms and insects, which are seized and swallowed alive. The frog uses his hands to help put the food into his mouth. The mouth has the sense of touch highly developed, but the sense of taste is present in only small degree. *In all cases, movement of a small living object gives the visual stimulus, and, psychologically speaking, brings to the frog the suggestion of something to eat.* Long experience of the race has taught that only immediate and swift motor response will result in capture of the food—the miller or grasshopper may take wing, the slug disappear under a board, or the caterpillar roll into a ball and "play dead." Usually, the result of the immediate seizure of the moving object is satisfactory, since *almost all small insects and worms are part of a toad's or frog's menu.* But sometimes lack of examination of the object brings dire results. Such is the case when a large stag beetle is swallowed. Its huge pinching mandibles produce terrible effect at once in the frog's stomach. Fortunately, the frog has a wide, short oesophagus, so that any disagreeable object can be disgorged immediately.

If frogs pursued their prey in the water as do fishes there would be no particular need for tongue, and some of the most aquatic frogs of the world have little or no tongue as a consequence. Most frogs, however, possess a thick, adhesive protrusible tongue,which is fastened at its forward end. The posterior end of the tongue can be shot forward and then quickly retracted with the prey affixed or held.

It is not necessary, however, to feed frogs which are being stored for testing purposes. Feeding is very difficult and unsatisfactory, so it is better to keep them under conditions where very little energy is used. When stored at the comparatively low temperature of 15° C. or below, as directed by the U.S.P., frogs are quite inactive and

require practically no food. It is necessary, however, to supply running water from which they presumably obtain sufficient nourishment.

Storage.—Frogs should be stored in tanks supplied with running water maintained at a temperature of 15° C. This can best be accomplished by means of a storage tank as described on page 208. The tank should be so constructed that the depth of the water is about one-half inch deep over one half of the bottom and about four inches deep over the other half.

The tank should be divided into several compartments so different lots of frogs may be kept separated. This is essential owing to the variation between different lots in their susceptibility to Ouabain.

The sides and bottom of the tank should be thoroughly scrubbed with a small brush at least twice a week.

Diseases.—The most common and deadly disease of frogs is known as red-leg. This was originally thought to be an infectious disease. It is no longer considered as such but is explained as being a capillary condition produced by too much activity under unnatural conditions or to other unfavorable conditions under which the frogs are kept. There is apparently no cure for the disease and all animals in which red-leg is noted should be immediately removed from the tanks and discarded.

GOLD FISH

Carassius Auratus.—The Golden Carp more commonly known by the name of Gold Fish owing to its golden hue, is a native of some of the ponds and sluggish streams of China. In their native waters, however, they are more of a silver color and do not have the bright golden coloring which is common to our usual aquarium varieties.

The Chinese breeders, who have for many centuries devoted all of their skill to breeding fine specimens, are to a great extent responsible for the much admired bright golden red color.

Gold fish are classified as to shape as Common Gold Fish, Japanese Fan Tails, Japanese Fringe Tails, Comets and Telescopes. Fine colors are found in all the classes,—golden red, silver, white, black and amber. These colors are often beautifully variegated.

Housing

The aquarium may consist of any water-tight vessel. Its shape is of little importance. The size depends upon the number of fish to be kept and whether or not the aquarium is supplied with aquaria plants or running water.

In aquaria without plants or running water a half gallon of water should be allowed for every fish of medium size. If the aquarium is supplied with growing plants one gallon of water is sufficient for four fish. If running water is supplied the number of fish per gallon of water may be greatly increased.

Aquaria without running water should not be placed in the sun as the water becomes warm and unsuitable for fish. Another objection to too much sunlight is that it accelerates the growth of algae.

Plants for the Aquaria.—If running water is not supplied, the aquarium should contain an abundance of aquaria plants. These plants in the presence of light, exhale oxygen which is required by the fish to maintain life. If the number of fish is not in excess of four to the gallon of water and there are plenty of aquaria plants present the water need not be changed except when it becomes necessary to clean the aquarium which should not exceed two or three times a year.

Various plants are suitable for growing in aquaria. The most common of these are the Umbrella Plant and Syperus, Colomba or Aquaria Moss, Ludwigia, Lemma or Duckweek and Sagitaria.

If plants are to be placed in the aquarium the bottom should be covered to a depth of about 1/2 inch with well washed sand. This should be covered with a layer of clean pebbles and a *few* shells. It is not advisable to use too many shells as the lime hardens the water.

After adding water to a depth of about 2 inches the plants should be inserted into the sand and weighted down until roots grow sufficiently to hold the plants in position.

Scavengers.—The properly stocked aquarium should contain some scavengers to consume decaying vegetable matter, and keep down as much as possible the growth of confervae. Snails, tadpoles and newts are used for this purpose. Snails and tadpoles subsist largely on the growing plants in the water but newts should be fed scraped raw beef, earth worms or insects.

Water.—Ordinary "tap" water, spring or well water is best. Cistern or distilled water should not be used.

The temperature of the water should be kept at about 60 to 75° F.

Feeding.—The best food for gold fish is the rice flour wafer or the mixed food containing dried meat sold under the name of Prepared Fish Food and Prepared Mixed Fish Food respectively.

Fish kept in running water may be fed raw beef or chopped fish worms. All food containing yeast such as bread, crackers, etc., must be strictly avoided. Care should be taken not to over feed. A piece of the prepared food 3/4 inch square per day is sufficient for a medium sized fish.

Diseases.—Very little is known about the diseases of fish but salt water has been proven to be a specific for many fish diseases. Salt water apparently acts as a tonic which by invigerating the fish enables it to throw off the disease.

The usual treatment for a sick fish is to place it in a shallow dish containing a solution of one teaspoonful of salt to a quart of water. When the fish floats at the surface from exhaustion (usually after about two minutes) it should be removed to a small aquarium or other vessel containing a solution of one teaspoonful of salt to one gallon of water. Repeat the above treatment with the stronger salt solution daily until the fish has apparently recovered.

Among the common diseases of fish are the so-called fungus, asphyxia, twitters, dropsy and bladder complaint.

As only the common cheap variety of fish are used for laboratory purposes it is more economical to discard fish afflicted with any of these diseases than to spend time trying to treat them.

GUINEA-PIGS

The Cavy, more commonly known as the Guinea-pig is a native of South America and belongs to the rabbit family.

The first white explorers of South America found that the Indians had domesticated the Cavy. Besides being used for food purposes they were kept as household pets.

The wild Cavy of South America are still hunted as game and considered a delicacy.

Although guinea-pigs are used as food and as pets, they are in demand in far greater numbers for laboratory purposes.

The most satisfactory size for testing purposes is from 250 to 500 grams which size they attain in from six weeks to six months. Full grown guinea-pigs, however, weigh from 675 to 1400 grams. Their growth is comparatively rapid as they attain approximately their maximum size in about eighteen months.

The origin of the name guinea-pig is apparently unknown. Some think it is perhaps due to the fact that their shape suggests a small pig and that the name Guinea is a corruption of Guiana, a country in South America.

There are three principal varieties of Cavies distinguished mainly by their fur,— the English, Abyssinian and Peruvian.

The Abyssinian and Peruvian are long ruffled-haired fancy breeds. The English or short smooth-haired cavies are the ones generally used for laboratory purposes. They come in a variety of colors, such as black, brown, cream, white, silver, gray, brindle, fawn or a mixture of these colors. The whites are usually albinos and have pink eyes.

Housing.— The size and style of the guinea-pig cage or cages depends primarily upon the number of animals to be housed. In our laboratory we keep these animals in multiple cages of the type shown in Fig. 87. Each unit of this cage accommodates about 25 pigs. While the animals are under test we use cages of the type shown in Fig. 86. Two to four pigs, of different color for identification purposes, are placed in each cage. The small cages are very serviceable as they may easily be carried into the laboratory where the animals are weighed and injected with the preparations to be tested.

The cages must be kept *dry* and clean. Guinea-pigs can stand low temperatures if the cages are dry and the animals are not in a draft. On the other hand they die rapidly if kept in a draft or wet cages.

The trays of guinea-pig cages should be covered with sawdust to a depth of about 1/2 inch to absorb moisture. On top of this a bedding of about three or four inches of hay should be placed. If the animal house is not heated during the winter months the hay helps protect the animals from the cold. At all seasons of the year the pigs will eat a considerable portion of the hay and the coarser parts make good litter.

The trays should be emptied at least twice a week and washed with an antiseptic solution before filling with fresh sawdust and hay.

In unheated houses the cages should be covered, during extremely cold weather, with burlap. This keeps out all drafts and at the same time allows sufficient air for breathing purposes.

Feeding.— Hay, grain and green foods form the principal diet for guinea pigs. Hay should be before them all the time. Grain should be fed daily during the winter but sparingly in summer. Green food should be fed the year round and varied as much as possible.

Of the grains, oats is the best although bran, wheat or rye serve the same purpose.

Guinea-pigs will accept almost any kind of greens such as grass, young wheat, alfalfa, clover, green corn stalks, melon rinds, apple peelings, cabbage, celery, kale, etc. Carrots and beets may also be classed as green food.

Green food should be fed sparingly during the winter and plentifully during the summer.

All green food must be in good condition. Spoiled, sour, musty or mouldy food will kill a large number of pigs. Decayed portions of green foods should be carefully removed before feeding.

Lettuce, celery and beet tops are among the doubtful foods. Too much of these greens seem to kill the animals. Also never feed grass or young wheat after it has been frosted or when it is wet with dew.

Do not overfeed as this is apt to cause bowel trouble. Do not feed at one time more green food than they can eat in an hour. They are not apt to overeat hay but will eat too much green food if it is kept before them all the time.

Best results are obtained by feeding twice a day, morning and evening.

Water.—Water should never be given while feeding green foods. When feeding only hay and grain, water is necessary. Change water daily.

Breeding.—Most laboratories find it more convenient and cheaper to buy their supply of guinea-pigs than to breed them.

For those who prefer to breed their own stock the following information is of value.

Guinea-pigs have from three to five litters per year with two to five in a litter.

Young females should not be bred until three months old.

The period of gestation is from 65 to 70 days.

The young are fully developed at birth and are usually able to run around within a few hours.

The young begin to eat green food within two to three days after birth.

The young may be weaned when two weeks old.

About five females and one male should be kept in each breeding cage.

For best results each female should not have over four litters per year.

Several females with their young should be removed from the males and placed in a cage together. The females nurse each others young as the young apparently cannot distinguish between the nursing mothers.

Female guinea-pigs for pituitary assays should be segregated at time of weaning and kept out of sight and smell of males.

Diseases.—Practically all diseases of guinea-pigs can be traced to improper care or food. As before stated damp cages, drafts and decayed food will cause the death of many pigs.

Bowel Trouble.—Too much green food especially in winter produces bowel trouble. In unheated houses this condition is also caused by sudden changes of the weather or insufficient bedding.

When suffering from this complaint the coat gets rough and stands on end. The animal appears humped and many times the disease kills within 24 hours.

Separate all sick animals, administer castor oil, supply thick bedding and disinfect cages.

Colds and Pneumonia.—These diseases are rare in properly heated and ventilated houses. In unheated and drafty houses, however, it is not uncommon to have the entire stock wiped out with epidemics of these diseases.

As these diseases are contagious all affected animals should be removed and the cages disinfected. The sick pigs should be placed

in warm cages and treated with a few drops of sweet spirits of nitre twice a day.

Lice.—Treat the same as dogs. (See page 243.)

Other Diseases.—Guinea-pigs are also subject to such diseases as Paralysis, Worms and Tuberculosis. As only the common cheap variety of guinea-pigs are used for laboratory purposes it is usually cheaper to kill animals suffering with these diseases than to spend the time necessary to try to cure them.

RABBITS

The rabbit is indigenous to virtually every part of Europe and America. Australia has also a species of rabbit somewhat akin in type and habits to the American "Jack."

The rabbit is a separate species from that of the hare. The rabbit is of the species *lepus cuniculus* while the hare is of the species *lepus timidus*. Both belong to the family *rodentiae* a creature with long rat-like front gnawing teeth.

In its gregarious habits the rabbit also differs widely from those of the hare, and in the wild state is said to be monogamous. This latter trait, however, ceases with domestication, and either sex becomes altogether polygamous. It also differs from the hare in that its young are born immature, with eyes closed and the body nude of hair, in a nest lined with fur pulled from the mother, burrowed in the ground whenever possible; while those of the former (hare) are born with eyes open, and body nicely covered, in a "form" on top of the ground.[1]

Varieties.—There are many varieties of domestic rabbits the more common of which are the American, Dutch, Lop, New Zealand, Polish, English, Angora, Spotted, Flemish Giant, Belgian Hare, The Tan in Black, the Silver in Brown, Fawn, Grey and Blue, the Havana, Imperial and Selfs.

Housing.—The cages shown in Figs. 87 and 91 are very satisfactory for housing rabbits. The bottom of the trays should be covered with sawdust on top of which is placed three or four inches of hay. If the rabbits are to be kept outdoors wooden hutches are

[1] Deardorff, Rabbit Culture and Standard.

preferable as they are warmer in winter. The character and size of the hutches depends upon the number of rabbits to be housed. Any style or shape box is apparently satisfactory. Five sides of the hutch should be constructed of wood. The sixth side should contain the door made of a wooden or metal frame covered with wire screen. For breeding purposes one end of the hutch should contain a nesting compartment, separated from the rest of the hutch by a wooden partition containing a hole sufficiently large to permit the rabbits to pass from one compartment to the other. Each compartment should contain a removable metal tray to facilitate cleaning. It is not necessary to heat outdoor hutches as rabbits can stand cold weather. The hutches should, however, be protected from drafts and storms in winter and excessive heat and direct sunlight during the summer. During extremely cold weather burlap or muslin covered frames should be provided to close openings in the hutches. These frames by reducing the rapid circulation of air, tend to retain the warmth generated by the animals bodies, and at the same time permit the escape of impure air through the fabric.

During cold weather the bedding of hay should be increased to a depth of five or six inches. This helps protect the animals from the cold.

All cages or hutches should be regularly cleaned and disinfected as described under "General Principles."

Exercise.—The rabbits should be placed in the cages or hutches in such numbers as to permit sufficient room for proper exercise. If too crowded the animals do not receive sufficient exercise to maintain a healthy condition.

Feeding and Watering. Rabbits should be fed and watered practically the same as guinea-pigs. (See page 253.) Dandelion seems to be injurious and plantain should be fed only sparingly. Turnips and carrots are a good relish now and then but hay-clover, alfalfa or timothy and oats should form the principle diet. Water should not be given when greens are fed. *Never give green food wet.*

Some prefer a mixed food containing cracked corn 10 per cent., homony 5 per cent., whole wheat 10 per cent., ground oats 20 per cent., whole oats 25 per cent., bran 30 per cent. To this should be added one half ounce of salt to each four quarts of mixed feed.

Breeding.—As with guinea-pigs most laboratories find it more convenient and cheaper to buy their supply of rabbits than to breed them.

For those who prefer to breed their own stock the following information is of value:

Rabbits have from six to eight litters per year with four to eleven to the litter.

The period of gestation is 31 days and if it varies more than one or two days longer something is wrong.

The doe will begin building her nest, and pull fur with which to line it any time from 10 days to an hour before due to kindle. It is necessary, therefore, to have short hay or straw present at all times for her use in building the nest.

A few days before a doe is due to kindle have before her continually a dish of clean, fresh water. Many does become feverish at this time, and will not infrequently destroy their young if the desire for water is not satisfied.

After the doe has kindled remove any dead from the nest.

Best results are secured by reducing the litter to six. Above this number calls for extra attention, food and care.

During the time a doe is suckling her young she should have constantly before her a dish of bread and fresh milk. Do not allow the milk to sour in the dish. The acidity of sour milk will cause what in rabbits is known as "slobbers" which is a very annoying trouble.

About five females and one male should be kept in each breeding cage.

For best results each female should not have over five litters per year.

Bucks become virile at about four months of age. It is best, however, to use bucks that are at least eight months old.

Does may be kept together in one compartment until bred but each adult buck must be kept in a separate cage or hutch as they are liable to kill each other by fighting.

When breeding it is advisable to take the doe to the buck's hutch.

Handling.—When handling rabbits *do not pick them up by the ears.* The proper method of handling is to grasp the loose skin over the

middle of the back with one hand and allow the rump to rest on the other. When carrying grasp as above and allow weight to rest on forearm.

Diseases. If proper attention is given to the housing and feeding of rabbits they are generally hardy and free from disease.

The diseases which are most common to rabbits are snuffles, slobbers and vent disease.

Animals suffering with *snuffles* should be isolated immediately and the cage or hutch thoroughly washed with a disinfectant solution before using for other animals. The treatment for snuffles is to place the animal in a warm cage well bedded with hay and administer two or three drops of tincture of aconite three times a day. If the animals are part of an experiment and thus of sufficient value to warrant the time and attention necessary it is advisable to daily pipette into the nostrils a solution of equal parts of Solution S-T-37 (Hexylresorcinol 1 1000) and water or some equally efficient non-toxic germicide.

Slobbers.—This trouble is apparently due to indigestion caused in most cases by the too liberal feeding of greens, wet or decayed greens or sour milk. This trouble should, therefore, be *prevented* rather than treated. It may be treated by administering the proper doses of any good digestive stomachic.

Vent Disease.—Animals infected with this disease, sometimes called vent gleet, should be immediately isolated and the cages disinfected. If the animals are of sufficient value inject daily with Solution S-T-37 or a 5 per cent. solution of Colloidal silver.

Other diseases to which the rabbit is susceptible include ear canker, diarrhea, paralysis, skin eruptions, constipation, etc. Very little can be done for these diseases and it is usually cheaper to discard afflicted animals than to try to cure them.

Fleas and Lice.—Same as for cats. (See p. 239.)

RATS

The albino rat (Mus norvegicus albinus) is the most satisfactory animal for vitamin assays and other nutritional investigations, since the effects of an exclusively vegetable diet or those of a strictly animal diet may be observed. The rat is to be preferred, therefore,

for metabolism experiments such as studies in dietary deficiencies and the biologic assays for Vitamins A, B, D, E and the P-P Factor. (See Chap. 13, p. 169.)

The rat is of especial value in many kinds of experimental work owing to the large number in each litter. This makes it possible to select both the control and experimental animals from the same litter and thus avoids variations which may exist between animals of the same age but from different litters.

Housing.- The albino rat is one of the cleanest of all laboratory animals if properly housed. Rats so housed are free from odor and there is no necessity for the nauseating odor so often prevalent where rats are stored. If the cages are properly constructed, cleaned semi-weekly, provided with fresh water daily and have an absorptive bedding, hundreds of rats may be housed in one room without any objectionable odor.

Cages constructed of metal are more durable and easier to clean and disinfect than wooden cages. The climate and the conditions of the experiment should govern to a marked degree the materials from which the cages should be constructed. Metal cages are to be preferred but should be placed in a well heated room away from drafts. The temperature of the room should range between 65° and 75° F. and *must not be damp.* Small metal cages such as used in vitamin assays should be placed on wooden shelves with hinged wooden fronts to help retain the animal heat and avoid drafts. The wooden fronts, however, should only be three quarters of the height of the space between shelves. *Breeding cages should always be constructed of wood* as the nests are frequently located so that the outer wall of the cage forms one side of the nest. All cages, whether constructed of wood or metal, should have bottoms made of wire screen with a metal tray underneath for holding sawdust. The front of the tray should be about 1/2″ longer and wider than the opening into which the tray slides so that when in position the front prevents drafts by closing the opening to the space beneath the wire screen floor.

Light and proper ventilation are essential. The animals generate a lot of heat and give off quantities of moisture which must be dissipated by proper circulation of air.

The cage shown in Fig. 86B is suitable for housing two to three full grown or six to eight small *stock rats*. The water is placed in the drinking fountain shown attached on the side of the cage. The bottom of the cage is of wire screen which allows the droppings to pass through into the tray beneath, which is one half filled with sawdust.

Each unit of the cage shown in Fig. 87 is suitable for housing twenty-five stock rats.

For best results the *breeding cages* should be of special construction with one compartment for nesting and another for exercising.

Very satisfactory cages for this purpose were designed at the Wistar Institute of Anatomy and Biology by Greenman and Duhring[1] which they describe as follows:

"For the production of the best animals two types of cages are desirable. First, a dormer cage and, second, an exercising cage. A revolving cage or a turntable is used for the latter purpose."

"The two forms of cages which we have adopted after considerable experimental work with several types of cages are here described."

Dormer Cage

"To distinguish them from other types of cages, like the exercising cage, the special cages used for experiments in nutrition, etc., we have designated as 'dormer cages' (Dormir, to sleep) those cages in which stock animals are bred, reared, and housed."

"The dormer cage is constructed of New England white pine 1/2″ thick, and is 35″ long, 12 1/2″ high, and 17″ deep, all outside measurements. It is divided in the middle by a partition into two compartments, each compartment measuring 16″ × 16″ inside, with a height of 8 1/2″ from the removable screen floor to the ceiling. The two compartments communicate through a circular opening 3″ in diameter, located near the rear of the cage. The object of this division is to increase the number of more or less sheltered positions where the rat may build its nest or protect itself from direct lights. It affords the rat an opportunity to escape from one com-

[1] Greenman and Duhring,—Breeding and Care of the Albino Rat for Research Purposes.

partment to the other if frightened. This simple shifting of location appears to satisfy the animal that it has protected itself. Furthermore, it is sometimes desirable to close the opening in the dividing partition in order to confine the rats in one compartment while the other is being cleaned."

"The cleats used in the construction of the cage, across the ends, and on the lower edge of the dividing partition are all without the cage, thus avoiding wood angles within the cage."

"The circular opening from one compartment to the other presents the only angle or edge within the cage which the rat may gnaw. This edge is protected by a metal band."

"Everywhere else within the cage the wood presents only flat, smooth surfaces which the ordinary albino rat will not attempt to gnaw. Special protection must be provided in cages which are intended to contain extracted albino rats."

"For the cage floor in each compartment, a removable galvanized wire-cloth screen 15 3/4 × 15 3/4" (no. 22 wire 1/8" mesh) having a folded edge 3/4" wide, of no. 22, galvanized sheet steel is provided. This screen floor is supported along the front and along the rear by 3/4" × 3/4" × 1/16" galvanized steel angles. Wood or fiber floor may be used if desired for protection against cold. Beneath the removable floor is a galvanized sheet steel drip pan or tray, 15 3/4 × 15 3/4 × 1/2" deep. This pan catches the drips and finer particles of dirt falling through the cage floor."

"At the right-hand end of each cage is the drinking fountain carrying a one-quart water bottle. This drinking fountain consists of a galvanized iron pocket opening into the side of the cage. Into this pocket projects the metal tube outlet of the inverted water bottle. A fresh drop of water always hangs on the slightly constricted outlet of this tube. Here the rat may drink, but it cannot foul the water supply. Furthermore, the outer end of this iron pocket carries a wire screen to admit light. This inhibits the rat from filling the space with food, litter, or other materials, and thus interfering with the proper working of the drinking fountain. The drinking fountain is so constructed that all excess water dropping from the water bottle flows out the end of the cage, thus preventing the wetting of the cage and the bedding on the cage floor."

"The two doors of the cage are made of galvanized wire cloth, 1/4" mesh, with a folded edge 3/4" wide of no. 22 galvanized sheet steel riveted at the corners. In the middle of one side a stove bolt with suitable brass sleeve and lock-nut furnishes both a handle for the door and a part of the locking mechanism. It is well to paint the wire-cloth door with some hard black enamel. This renders the contents of the cage more plainly visible."

"The doors are not hinged to the cage, but set into the front openings of the cage, so as to protect all exposed edges of wood and so they may be easily removed. In feeding, the caretaker may unlock and open a cage door with one hand while serving food with the other."

"At the rear of the cage and along its entire length at the upper part, a 1 1/2" opening covered with 1/4" mesh galvanized wire cloth affords ventilation for the rats."

"The cage is so constructed that one may set upon the top of another in banks of four or five as may be desired. No projecting part interferes with this arrangement. Or they may be supported on a rack with projecting arms which would come just under the overhanging upper edges of the ends. With such a supporting rack, each cage may be removed without disturbing others."

"Our own practice is to pile the cages five high on an angleiron frame suspended from the ceiling so that the lowest cage is 14 inches from the floor."

"The light weight of such cages makes it possible for a person with a small amount of physical strength to handle cages. Such a cage, stripped of its accessories, weighs 14 1/2 pounds."

"In cage construction the economy of operation should be considered if there are many cages in service. Light-weight cages can be cleaned and sterilized more quickly than heavy ones. The lighter the cage, the less heat units will be required to sterilize it."

"The dormer cage here described is intended to accommodate one breeding pair of rats and a litter of young. Not more than eight or ten adult albino rats should be kept in a cage of this size."

The construction of the dormer cage with a turntable exerciser in place is shown in Fig. 94.

Exercising or Revolving Cage

"The exercising cage is an essential part of the colony equipment if fertility is to be maintained and vigorous rats are desired."

"The cage which we have found very satisfactory is constructed upon a 21-inch bicycle wheel. The excellent ball bearings of a bicycle wheel are essential, for revolving cages are subjected to a

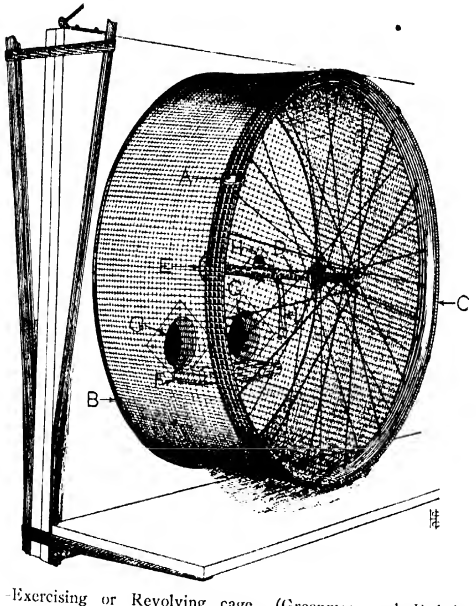


FIG. 92.—Exercising or Revolving cage. (Greenman and Duhring, *The Albino Rat.*)

very considerable daily use. The recording mechanism frequently registers 5000 revolutions in the twenty-four-hour period."

"Each revolving cage is provided with a galvanized steel pan, 18″ × 10″ × 1/2″ deep, so placed on a shelf beneath the drum as to catch the drips and refuse falling from the drum. Most of this material passes out between the free edge of the drum and the background."

"The nest box (Fig. 93), located on the rear face of the background is 21 1/2" long, 8 1/2" high, and 7 1/2" wide, and is constructed of 1/2" thick white pine. It is divided in the middle by a solid wood partition into two compartments. Each compartment communicates with the revolving drum through a 2 3/4" circular opening, already shown in Fig. 92 (*G,G*). The nest is accessible through the top, which is closed by a lid extending its whole length. In the lid over each compartment a 2" circular opening covered by a wire screen affords ventilation for the nest box (Fig. 93*A*)."

"One side of the nest box is closed by the background which carries it. Across this side of the nest box, a 1/2" × 18" flat iron bar (*B*) is screwed to the ends and middle of the box, and is utilized to carry the box on two flat hooks, secured to the background. This arrangement makes it possible to remove the nest box easily for cleaning."

"Each compartment of the nest box is supplied with a wire-screen floor (*C*), similar to the floor in the dormer cages. These are removable.

"Beneath the screen floor a galvanized sheet-steel pan (*D*) serves to catch the drips and particles of dirt falling from the nest box. Above the nest box is located the water bottle (*E*), with its extra long metal tip leading through the background into the revolving drum. The bottle rests upon a small bracket and is held in a vertical position by a wire loop at the top. The label holder (*F*) is fixed on the rear face of the background."

"We have used the revolving cages mounted singly on individual stands and in batteries of twelve or less mounted on one large background."

"The revolving cage here shown is one of a series of twelve mounted in a double row on a vertical background suspended from the ceiling. Beneath each row of six cages is a projecting shelf 10" wide to carry the drip pans under the cages."

"*The Turntable.*—As a less expensive substitute for the revolving cage, we have used the turntable mounted in a dormer cage. Its position and construction is shown in Fig. 94. The turntable consists of a wooden disc 1/2" thick and 14" in diameter."

"A modified bicycle wheel hub and axle (*A*) are used to carry the revolving table."

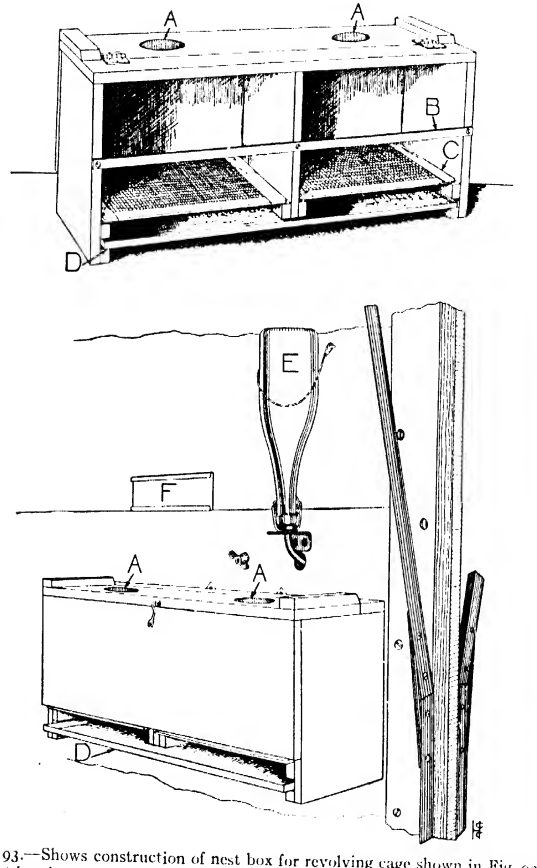


FIG. 93.—Shows construction of nest box for revolving cage shown in Fig. 92. The nest box is attached to one side of the background and the revolving cage to the other side. (Greenman and Duhring, *The Albino Rat.*)

"Secured to the lower end of the bicycle wheel hub is a brass disc 4" in diameter and 1/8" thick which carries the larger wooden disc."

"The upper end of the axle is provided with an elbow-joint (*B*), the position of which is controlled by a bolt and wing-nut permitting the turntable to be adjusted to any desired angle."

"A stud bolt (*C*) projecting from the upper portion of the elbow-joint passes through the ceiling of the cage and, held in place by a

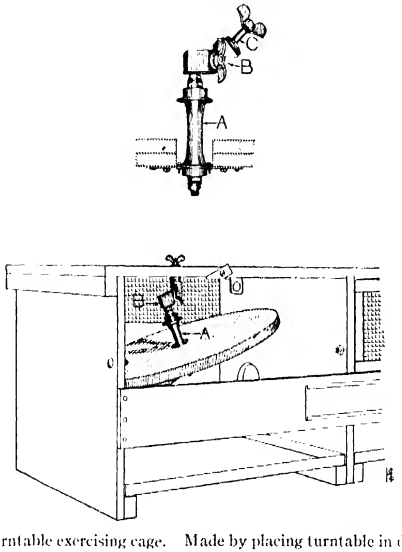


FIG. 94.—Turntable exercising cage. Made by placing turntable in dormer cage. (Greenman and Duhring, *The Albino Rat.*)

wing-nut, acts as a mounting for the turntable. By removing the wing-nut at the elbow-joint the turntable may be easily removed for cleaning purposes."

"While the turntable occupies considerable space within a cage and makes cage cleaning a little more difficult, we are inclined to the belief that it stimulates the rats to a greater variety of exercises than the revolving cage already described."

"A female appears to appreciate the advantages of the exercising wheel. When her pups are able to go out into the wheel, she will operate the wheel while they cling to the wire of the revolving drum."

"Occasionally a female will carry her pups from the revolving cage to the nest box. This happens when the pups are twelve to fifteen days of age. She apparently thinks they are too young for exercise. Later the female will sometimes take one pup at a time in her mouth and run with it in the revolving drum, then return this one to the nest box and take another out for similar treatment until the entire litter has been with her in the revolving wheel."

A satisfactory *cage for vitamine assays and other metabolism experiments* is shown in Figs. 68 and 67 p. 172. These cages are designed for experiments in which each rat is kept in a separate cage. For experiments where it is possible to keep three or four rats together the cage shown in Fig. 86 p. 232 is very satisfactory.

Feeding.- The albino rat thrives on almost any food utilized for human consumption. The more varied the diet the more vigorous the rats. The common foods which have been found to be acceptable to rats include fresh and powdered milk, whole wheat, whole wheat flour, cracked corn, rolled oats, corn meal, hominy grits, dried peas and beans, egg powder, salt, practically all kinds of vegetables and fruits such as oranges, apples and pears.

The mixed "scratch feed" supplied by poultry dealers for chickens contains a variety of food substances such as wheat, oats, cracked corn, barley, kaffir, milo, buckwheat and sunflower seed upon which the rats thrive. "Poultry Growing Mash" is also a very satisfactory food for rats. The more common brands contain dried buttermilk, oatmeal, corn meal, ground oats, wheat middlings, wheat bran, meat scrap, bone meal and fish meal.

Although a variety of food seems preferable, a mixture of Whole Wheat Flour 2 pounds, Powdered Whole Milk 1 pound and Sodium Chloride 1/2 ounce includes all the necessary elements for sustaining life. This mixture is satisfactory for normal growing and adult rats, lactating females and young pups. The animals remain thrifty, grow rapidly and can be fed indefinitely upon this mixture alone.

Breeders and others who maintain large colonies should use more varied diets. Complete instructions for the preparation of varied

diets have been published by Greenman and Duhring.[1] For vitamin assays the following diets are satisfactory:

Vitamin A-free Diet.—See p. 182.

Vitamin B-free Diet.—See p. 187.

Vitamin C-free Diet.—See p. 192.

Vitamin D-free Diet.—See p. 193.

Vitamin E-free Diet.—See p. 197.

Water.—Fresh clean water should be available at all times. The rats will quickly foul water exposed in open vessels. Some breeders, therefore, supply water only in drinking fountains while others maintain that the rats keep themselves much cleaner and the odor is less marked in the colony house if the water is supplied in large open dishes in which the rats may bathe. If the latter method is used the water should be changed twice daily. The large open water container seems preferable in summer as the rats bathe frequently and apparently appreciate the cooling effect of the evaporation of the water from their bodies. This is demonstrated by the fact that the nursing mother will ofttimes quickly dip each of her pups in the water and carefully replace them in the nest. If the tops of the cages are made of wire screen the adult rats will ofttimes take a bath and then hang from the top of the cage by their teeth until dry. In extremely hot weather a small cake of ice placed in the water container is of value. The rats nibble off small pieces and seem to enjoy the procedure as heartily as all small boys enjoy taking small pieces from the neighborhood ice wagon.

During feeding experiments with known or unknown vitamines the water *must* be supplied in fountains. In such experiments only *distilled* water should be used and a few drops of tincture of iodine should be added to each litre to prevent the growth of bacteria, wild yeast, etc. Ordinary drinking water may contain extraneous mineral salts, bacteria, algae, wild yeasts and vitamines which may entirely upset the entire feeding experiment. See p. 176.

Handling.—"As in the case of other domesticated animals, it is not sufficient merely to feed and water albino rats and keep their cages clean."

[1] See footnote on p. 260.

"Individual attention, shown by handling and petting, is essential for the best growth of albino rats and for securing uniform reactions when used as research animals. They should have ample opportunity to know their caretakers. Contentment soon replaces fear when they are placed in cages where they may receive some individual attention and where they may observe the various activities of the colony house, become accustomed to the noises of the place, and hear the voices of those engaged in the work of colony. Under such conditions, they eat and assimilate food in a more satisfactory manner. The absence of fear permits them to feed with pleasure and to digest food with none of the inhibiting influences of nervous tension which tend to restrain digestive secretions. Under such conditions, fertility is markedly increased."

"In handling rats, especially when they are accustomed to such treatment, one should approach the animals very slowly and quietly making no quick or unexpected movements."

"Do not pick up a rat by its tail if this can be avoided."

"Grasp the rat gently about the body with its head towards the palm of your hand, closing the fingers under the abdomen, with the little finger under the rat's jaw. Any attempt of the rat to bite you may then be thwarted by closing your fingers firmly on its throat."

"Once held in your grasp for a moment or two, the rat will relax and very soon become quite contented in your hands. After such daily treatment for a brief period the rat will display no fear and will be quite satisfied to be handled. Albino rats are likely to exhibit greater fear when approached in the darker retreats of their cages. They seek shelter in such retreats and resent any attempt to remove them. The same animals may be approached with less risk of offense when they are out in open space." (Greenman and Duhring.)

Breeding.—If only occasional vitamin A assays are made it is preferable to buy the necessary animals from breeders who maintain large colonies. This is necessary in order to meet the U.S.P. requirements as to age and weight. Laboratories in which all kinds of vitamin and dietary deficiency experiments are conducted find it more satisfactory to breed their own animals in order that they may be certain as to the animals belonging to the same litter, the exact age

of the animal, etc. For those who wish to breed their own animals the following information is of value:

The span of life is about three years.

The female mates when about eighty days of age.

The period of gestation is from twenty-one to twenty-three days.

The female may mate within two days after casting a litter.

One female may produce one hundred pups in a year.

Brothers and sisters may be mated with no unfavorable results.

The female usually ceases breeding when about 450 days of age.

Healthy females average seven to eight pups per litter.

One male may be caged with two or more females.

Heat or rut takes place every five days.

The male should not be removed from the cage when the female becomes pregnant or after the litter is born.

The nursing litter should be removed just before the succeeding litter is born.

Diseases.—The most common diseases of the albino rat are pneumonia, middle-ear disease and various deficiency diseases.

The deficiency diseases may be cured by supplying the proper diet. The best remedy for the so called pneumonia is hot cocoa administered every hour by means of a pipette.

Rats suffering with middle-ear disease usually carry the head turned slightly to one side. All such rats should be eliminated as they do not represent average normal rats and are, therefore, unsatisfactory for most research experiments.

Parasites.—Fleas and lice are the most common of the parasites which annoy rats. They should be treated the same as described under Cats. See p. 239.

ROOSTERS

(Cockerels and Cocks)

A *cockerel* is a male fowl less than *one* year old while the term *cock* is applied to a male fowl one year or more old.

There are many breeds of cocks only one of which (the White Leghorn) is official for Biologic Assay purposes.

The leghorns belong to the Mediterranean class which had their origin in countries bordering on the Mediterranean Sea. There are

eight varieties of Leghorns, as follows: Single and Rose Comb White, Single and Rose Comb Brown, Single and Rose Comb Buff, Single Comb Black, and the Silver.

The official cock for U.S.P. ergot assays is the *Single Comb White Leghorn,* less than eighteen months of age, and weighing approximately two kilograms.

The Leghorns were originally imported by America from Italy.

Housing.—For *cocks kept indoors* the cages shown in Fig. 91 are very satisfactory both for housing stock fowl and for caging during the fasting and test periods. Two units of the cage, without the partition between are used for housing each five cocks. Two roosts are placed across the end of one of the units. This allows sufficient room for exercise between tests. In order to prepare for the fasting period all of the cocks are placed in the one unit and the partition inserted between it and the other unit. The tray is then removed, emptied, washed and about 1/4 filled with clean sawdust. The partition is then withdrawn and the cocks driven into this unit. The partition is replaced while the tray of the other unit is emptied and the sawdust renewed. The partition is then removed. By this process the possibility of the cocks obtaining food from the bedding is entirely eliminated. During the fasting period the mash hoppers are closed. If only one or two of the cocks are to be injected at a time the partition is again inserted in order that those injected may be separated from the uninjected ones which greatly facilitates their removal for observation during the test period.

For *cocks kept outdoors* it is necessary to build some type of poultry house. The location and design of the poultry house and its construction determine in a large measure the condition of the cocks. A loose, dry, sandy, quickly draining soil is to be preferred. Avoid if possible heavy clays or land where rain water stands in puddles.

The bio-assayists, however, usually have practically no choice as to where they can keep their fowls. It is in most cases the college or factory yard. The principle condition, therefore, is the proper construction of the house. The essential requirements are comfort to the fowls and convenience for the attendant. It should be dry, cheerful, well ventilated, free from drafts, warm in winter, cool in summer, and easy to clean and keep in a sanitary condition.

Cocks have a relatively high body temperature (106 to 108°) and are, therefore, very sensitive to drafts. The best type of house for preventing drafts have the back and two ends closed with a properly constructed open front. The open front insures sunlight which in warm climates can be left open all the year and in cold climates the openings may be fitted with frames covered with burlap or unbleached muslin. This allows free passage of air but breaks up drafts.

The *floor* may be of concrete, cinders, earth or boards and should be a foot or more above the ground level to insure dryness. Dryness is one of the first essentials of a poultry house. Cold wet floors result in a very humid, unhealthy atmosphere. Cement floors should be covered with 4 to 8 inches of dry litter.

Sunlight is essential and it is important, therefore, that the house contain windows, preferably in the south side.

Eight to twelve square feet of floor space per bird should be allowed in small flocks and not less than four or five square feet per bird in larger flocks. Where birds are kept in cages in a large animal house the space per bird may be materially reduced.

Roosts.—About 50 per cent. of the cocks time is spent roosting and it is, therefore, important that the roosts be properly constructed. Very often the mistake is made of constructing roosts of lumber 1″ × 1″ or 1″ × 2″. Roosts should be made of 2″ × 2″ or 2″ × 4″ material with the upper corners slightly rounded.

Feeding.—Cocks for ergot assays whether housed in cages indoors or kept in outdoor houses should be fed mixed scratch feed, growing mash with buttermilk, greens, charcoal, and grit. These materials may be obtained from dealers in poultry supplies. The *growing mash* contains dried buttermilk, feeding oat meal, corn meal, ground oats, wheat middlings, wheat bran, meat scrap, bone meal and fish meal. The *scratch feed* contains wheat, oats, cracked corn, barley, kaffir, milo, buckwheat and sunflower seed.

The growing mash, charcoal, and grit should be before the cocks at all times except during the starvation period immediately preceeding each assay. These materials should be placed in 3-compartment *hoppers* constructed as shown in Fig. 88B. Hoppers for outdoor houses should be provided with a drop lid which should be

closed during the night. Open mash hoppers attract rats which will consume large quantities of mash.

The *function of the mash* is to supply proteins and fat in a form which is quickly and easily digested. When feeding dry mash always keep the supply of drinking water close to the mash hopper.

The *function of the scratch feed* is to supply carbohydrates. These supply body heat and induce the cocks to exercise. The scratch feed should be scattered in the litter so that the birds will exercise in searching for it.

Green food should be part of the daily diet of all caged or housed birds. Grass clippings, oat sprouts, celery tops, alfalfa, young wheat, clover, red beats, etc., are very acceptable.

Grit is a valuable aid to digestion. Cocks have no teeth and the grit is necessary in order that it may aid in grinding up the food in the gizzard.

Charcoal has been found to be a valuable or perhaps necessary adjunct to the diet of fowls.

Feeding Methods.—The quantity of feed to be given depends largely upon the appetite and number of the flock. In the morning only sufficient scratch feed should be scattered in the litter to induce the birds to come down off the roost and keep them busy for an hour or two. If fed too much in the morning they will sit around for many hours while the feed is slowly digested.

If greens are to be fed give about noon and open the mash hoppers so they may help themselves during the afternoon.

Late in the afternoon give sufficient scratch feed so that each cock will go to roost with a full crop. If a little grain remains in the litter, it will be scratched out early in the morning and keep the cocks busy until feeding time.

Moisture.—Although the house must be free from dampness a small percentage of moisture must be present or the birds will suffer from their legs becoming too dry. When they are kept in indoor cages it is advisable, once a day, to slightly dampen the sawdust with water by means of a small sprinkling can such as used for watering flowers.

Beak and Nails.—As stated in the introduction, the beak and nails of caged birds will sometimes become so long and curved

that it is difficult for them to walk or eat. It is necessary, therefore, at regular intervals to clip the beak and nails with bone clippers.

Water.—A supply of fresh water should be kept before the birds at all times. It should never be given in open dishes or hoppers. One of the commercial sanitary water containers should be employed. Open water dishes and hoppers are one of the greatest sources of spreading disease.

Sanitation.—If the cockerels are to be kept free from parasites and disease the most sanitary conditions must be maintained. Rational sanitary measures must be taken to prevent or delay the appearance of disease, to limit its spreading and to eradicate a disease which has already gained foothold. Cages should be cleaned at least semi-weekly. The trays should be scalded and washed with an antiseptic solution. The entire cage should be steamed or washed with an antiseptic solution at least once a month. Outdoor houses should be sprayed. Tray supports in cages and all cracks and crevices in houses should be sprinkled with a mixture of Sodium Fluoride 50 per cent. and Sodium Borate 50 per cent.

Parasites.—Parasites by their irritation and blood sucking lower the vitality of the cocks and make them more susceptible to disease. The common external parasites which infest cockerels are *lice and mites*. Lice usually live, breed and have their complete cycle on the bird whereas mites usually originate elsewhere and go to the birds for feeding. When found dust the birds with a mixture of Talcum 60 per cent. and Sodium Fluoride 30 per cent. Disinfect and if possible sterilize all cages or houses. The author has been able to entirely prevent infection by using cages as shown in Fig. 91 which are sterilized once a month with live steam applied by means of a hose.

Scaly legs is a condition common in some localities so called because of the accumulation of scales or scabes upon the legs. This is a form of scabes caused by the parasite Sarcoptes mutans, variety gallinae. This condition should be treated by soaking the scabs with warm water and removing as many as possible by means of a small brush. Apply antiseptic solution.

Caged birds if kept under proper conditions are seldom troubled with the internal parasites such as round worms, gizzard worms, gape worms and tape worms.

Diseases.—Cockerels are susceptible to the following diseases: The non-contagious diseases include abstruction of the beak, pip, sore mouth, diseases of the crop, limber neck and colitis. The contagious diseases include blackhead, fowl cholera, white diarrhea, tuberculosis, chicken-pox and roup.

In bio-assaying laboratories cocks are used only for ergot assays. The birds are, therefore, of little more value than their purchase price. It is usually more advisable, therefore, to discard afflicted animals than to try to cure them.

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CHAPTER XVIII

ARRANGEMENT OF LABORATORIES[1]

The arrangement of the biologic assay and research pharmacologic laboratories of the pharmaceutical manufacturer is of the utmost importance if quick efficient service is to be rendered. The laboratory should be equipped with sufficient permanently set-up apparatus to make it possible, if necessary, to start routine biologic assays at a moments notice. The main laboratory should also contain a chemical table and a conveniently located case containing all of the necessary instruments and apparatus for conducting the various pharmacologic experiments.

The directors office, files, laboratory for conducting assays upon intact animals such as the assays for Ergot, Cannabis, Vitamin and other metabolism assays, the room for stock animals, and the room for animals under test should be located with the object of conserving time and space.

For general appearance, cleanliness and light reflection the walls, partitions, cupboards, work tables, apparatus cases, etc., should be of white enamel finish. Battleship linoleum is the most satisfactory floor covering.

The author prefers partitions only seven feet high in order that there may be a good circulation of air during the summer months. Paneled partitions in which the bottom panel is constructed of one piece of thick composition board 32″ wide and 48″ high and the top panel of one pane of glass 32″ × 31″ make a fine appearance. The single piece solid bottom panel lends itself to a white enameled finish and the glass top panels add greatly to the general appearance of the laboratories and the diffusion of light.

Partitions of this type are built as follows: The framework, both horizontal and vertical, is made of 2 × 3″ clear lumber planed on all

[1] The illustrations in this chapter were made from photographs of the author's laboratories at Sharp & Dohme, Baltimore, Md.

four sides. At all corners and the intersection of partitions the uprights are of $3 \times 3''$ material. The composition-board lower panels and the glass in the upper panels are held in place by $5/8'' \times 7/8''$ molding. The best affect is obtained if the molding for the glass is of a slightly different pattern than that used in the lower panels. The glass should not be placed in frames but simply held in position by the molding. This permits a much greater glass surface than if frames are used which, to the authors mind, greatly improves the appearance of the partitions and admits more light to the laboratories.

By standardizing on panels $32''$ wide it is possible at any time when alterations may be necessary to relocate doors without changing the framework. By removing the upper and lower panel a $32''$ opening is left which, after adding the $1''$ door frame on both sides leaves an opening for a standard $30''$ door. The use of this size panel also reduces the labor involved in building the partitions as the composition board is supplied in standard sheets $48 \times 96''$. Therefore, by simply making two cuts one piece will make three $32 \times 48''$ panels.

The top of the partitions are finished by the use of $3''$ crown molding placed on both sides. The groove between the two pieces of crown molding may be used to conceal gas, water and air lines running to the work tables. This avoids the necessity of hanging the pipes from the ceiling which greatly detracts from the appearance of the laboratories. Doors should be of the two panel type with a single wooden panel below with glass above. The height of the door panels should be the same as that of the partitions. Glass door knobs are preferable to metal as they are not affected by acids or other corrosive substances which may adhere to the operators hands.

Fig. 95 shows a floor plan of a very satisfactory arrangement of the various laboratories and general view of the authors research laboratory.

On one side of the **main or research laboratory** should be placed a *chemical work table* where chemical procedures incidental to the work of the laboratory may be carried out. (See Fig. 96.)

This table should have either a stone or acid finish black oak top $37''$ from the floor and be provided with cupboards, drawers, shelves

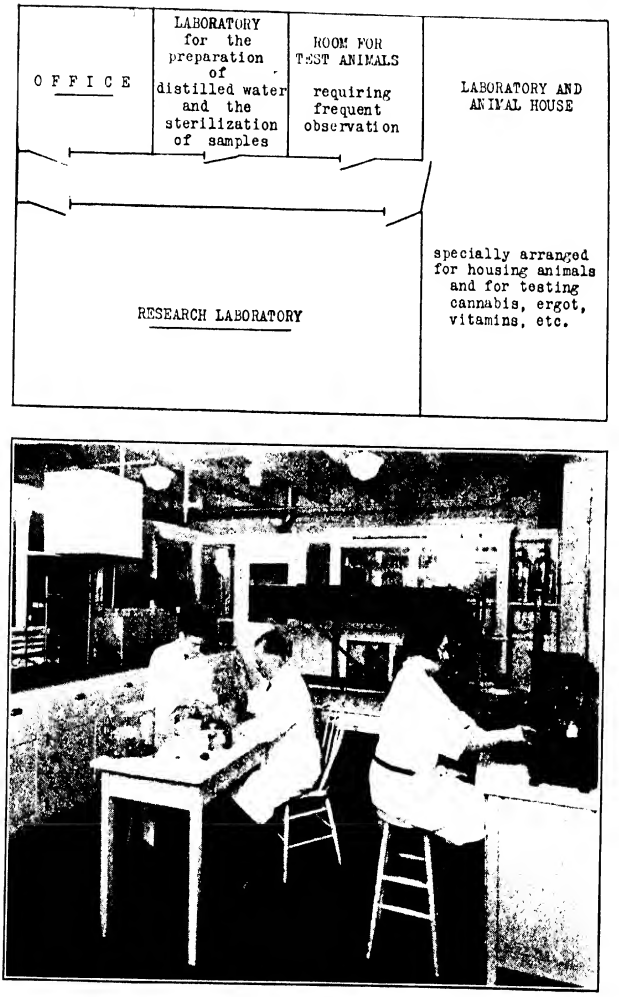

FIG. 95.—*Upper*, floor plan for biologic assay laboratories, office, animal rooms, etc. *Lower*, general view of author's research laboratory.

for reagent bottles, vacuum pump, gas, air, water, steam, electricity and convenient drains.

If the table top is finished in black the same finish should be used on the reagent bottle shelves, peg boards, animal boards, boxes for supporting apparatus, etc. This harmonizes well with the white enamel finish of the other woodwork.

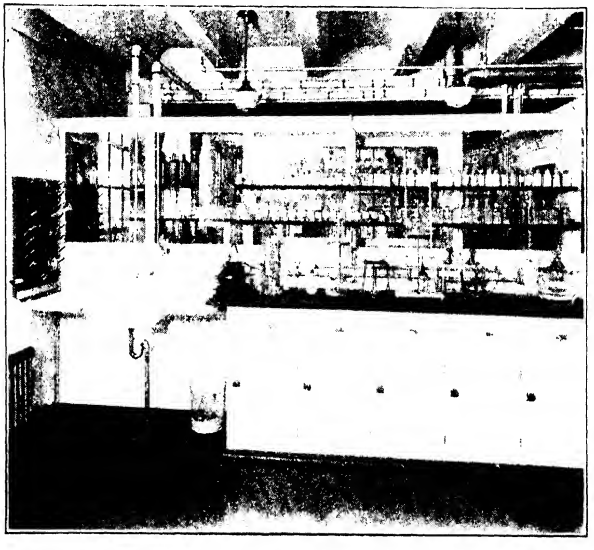

FIG. 96.—Chemical table with auxiliary apparatus, sink, peg board, etc.

A black acid finish for this purpose may be applied as follows:

Prepare a solution of Potassium Chlorate 125 grams, Copper Sulphate 125 grams and water sufficient to make 1000 c.c. Heat to boiling and apply two coats while hot. As soon as this is absorbed apply two coats of a solution of Aniline 120 c.c., Hydrochloric Acid 180 c.c., and water sufficient to make 1000 c.c. The second solution may be applied cold. Allow to dry for twenty four hours. Scrub with soap and water and again allow to dry. Rub in two or three

generous applications of linseed oil. Wipe off any oil not absorbed after several hours. This produces a coal black finish which is not materially affected by acid, alkali, alcohol, ether, etc.

The most convenient location for the sink and peg board is beside the chemical table as most of the glassware and other utensils are soiled by the chemical operations. The top of the sink should be about 33" from the floor. The peg board should be hinged at the top and equipped with a wooden prop which makes it possible to raise the bottom of the board to a height sufficient to make the pegs stand at right angles with the floor. After washing and placing the glassware on the pegs the prop is placed in position which allows complete drainage from flasks, etc.

On one of the other sides of the laboratory should be placed a *table for permanent apparatus*. This table may be constructed essentially the same as the chemical table except that the author in this case prefers a battleship linoleum top with brass edging. Cupboards and drawers for the storage of apparatus should extend the entire length of the table. Some of the cupboards should contain one shelf and others two shelves in order to accomodate various sized apparatus. Solid, polished brass hinges, catches and pulls contrast nicely with the white enamel finish. This table should also be equipped with gas, steam, vacuum, air, water, electricity and drain with convenient outlets and stopcocks for each. (See Fig. 97.)

This table should hold all apparatus for which there is sufficient use to render it advantageous to be kept permanently set-up. The table shown in Fig. 97 holds the steam bath, electric oven, electric stirrer, constant temperature bath for frog assays and the apparatus for testing drugs upon the isolated uterus.

Against the third side of the laboratory may be built a large case with sliding glass doors, for holding operating instruments, heart and muscle levers, tambours, manometers, oncometers, cannulae, inductorium, moist chambers, electrodes, etc.

For this purpose the author prefers a case built the entire height of the partitions (7 ft.), divided into four sections—two high and two wide. The two upper sections may be equipped with three plate glass shelves for holding operating instruments and small apparatus. It is preferable to use wooden shelves in the lower

sections in order to support the heavier apparatus such as the stands, supports, etc.

Shelves should also be placed in a convenient location for holding the bottles containing the stock solutions such as Saline Solution, Locke-Ringer Solution, etc.

On the fourth side of the laboratory may be placed the operating table with holder for small animals, the balance table, the smoking and fixing stand and the table with large animal holder and board.

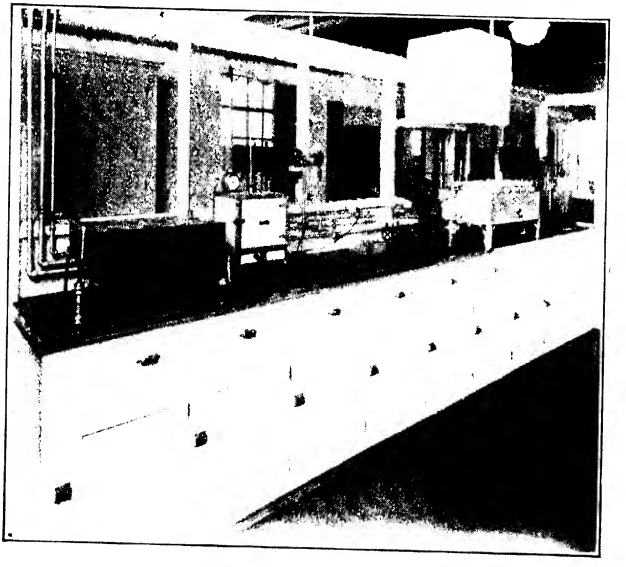

FIG. 97.—Table for permanent apparatus.

It is usually most convenient to place the *kymograph and auxiliary apparatus* in the center of the laboratory. Fig. 98 shows the long paper kymograph with manometer, time recording device, signal magnets, plethysmograph, animal board, artificial respiration apparatus, etc., in place for conducting an experiment.

A storage tank for air equipped with reducing and automatic blow-off valves to maintain an air pressure of four pounds may be

attached to the ceiling directly over the kymograph for use in experiments requiring artificial respiration.

It will be noted that a research laboratory of the above description is equipped to carry out U.S.P. frog assays; assays upon the isolated uterus, intestinal strips, etc.; tests upon the blood pressure, respiration, kidneys and heart; oral, subcutaneous and intravenous toxicity tests, etc.

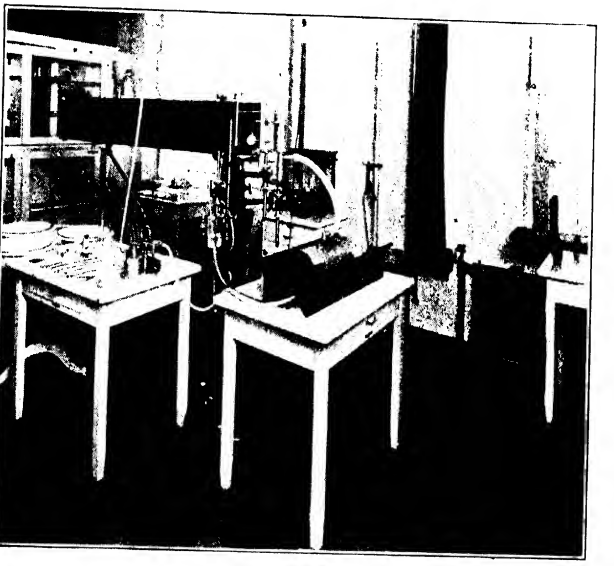

FIG. 98.—*Center*, kymograph with auxiliary apparatus. *Background, left*, instrument case with sliding doors. *Background, right*, operating table with small animal board, balance table, smoking and fixing stand and operating table with large animal board.

Instead of bringing the animals into the main laboratory for cannabis, ergot and vitamin assays, the standardization of local anesthetics, mydriatics, myotics, etc., it is preferable to conduct such assays in a specially equipped **combination laboratory and animal house.** (See Fig. 99.)

The cages shown at the left of the illustration house the dogs for cannabis assays and the cocks for ergot assays. These cages are separated by removable partitions and each has a removable sliding tray which acts as a bottom for the cage. The partitions separate the dogs so they cannot see each other during cannabis assays while the perforated metal fronts make it possible to observe the action of the drug upon the animals without removing them from the cages.

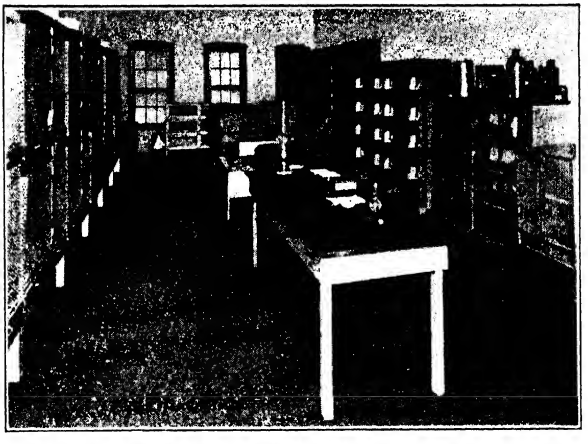

FIG. 99.—Large laboratory and animal house specially equipped for ergot, cannabis and vitamin assays.

The removable trays are especially convenient for ergot assays. They are removed, washed and covered with clean sawdust just preceeding the fasting period. This eliminates the possibility of the cocks obtaining food from the litter or the necessity for placing them in other cages during the test.

The cages shown at the rear of the laboratory are for breeding and housing stock albino rats and the cages on the right are for cats used for testing mydriatics and myotics.

The shelves on the right are specially constructed for holding individual metal metabolism cages for vitamin assays.

For a detailed description of the construction of the various cages, shelves, etc., see "Care of Animals" Chapter XVII and "Vitamins," Chapter XIII.

There are many experiments such as the determination of the effects of hypnotics, toxicity tests, metabolism experiments, etc. in which it is necessary for the operator to observe the condition of the

FIG. 100.—Room for housing test animals requiring frequent observation.

test animals at frequent intervals. It is very convenient to have a small room specially equipped for this purpose. (See Fig. 100.)

Such a room should contain small, medium and large cages. The latter should be equipped for metabolism experiments. This room should also contain a large metal combination sink and sterilizer. The sink shown at the left in the above picture is equipped with

hot and cold water and live steam. The cages, after washing, may be placed in such a sink, a metal cover clamped on and live steam admitted to sterilize the cages.

A small laboratory for the preparation of distilled water, solutions for assay and the sterilization of solutions and containers is a valuable adjunct to the laboratory.

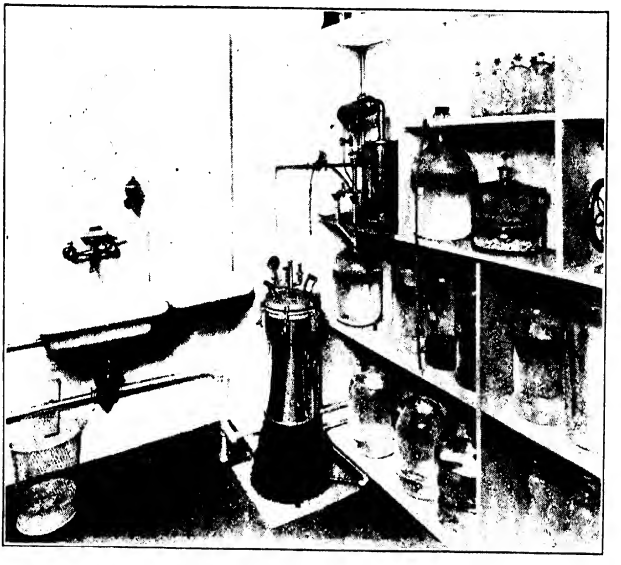

FIG. 101.—Small laboratory for the preparation of solutions and distilled water, sterilization, etc.

Quantities of freshly distilled water are required for the preparation of Locke-Ringer's Solution for isolated uterus experiments, for watering rats used for vitamin assays and for many other routine laboratory procedures.

Figure 101 shows the arrangement of such a laboratory.

Along one side of the room shelves are placed for holding the Barnstead Still, receiving bottles and the various sized pyrex bottles

in which distilled water and stock solutions are stored. The top shelves hold one litre, the middle shelves nine litre and the bottom shelves five gallon pyrex bottles.

One of the difficulties encountered with the use of an automatic water still is the frequency with which the receiving bottle becomes filled in the absence of an attendant with resultant overflowing. This is avoided in our laboratory by the arrangement shown above. The still is placed on the third shelf from the floor. The water from the condenser flows through a tube which passes through and extends to about 1″ below a tightly fitting stopper in the five gallon pyrex bottle on the second shelf. A siphon tube passes from the bottom of this bottle up through the cork and down through the shelf into another five gallon pyrex bottle on the shelf below. The stopper of the upper bottle contains a third hole through which passes a tube connected with an atomizer bulb. The opening around the tube passing into the lower bottle is filled with cotton. Should the attendant notice that the upper container is almost full the siphon may be started by pumping air into the bottle by means of the atomizer bulb. Should the upper bottle not be emptied before it is full it will automatically empty itself through the siphon tube. The lower bottle as soon as filled should be replaced by an empty bottle.

A small autoclave is necessary for experiments involving the determination of the effects of sterilization at various temperatures upon the activity of preparations intended for hypodermic injection, etc.

The opposite side of this room should contain a work table equipped with the apparatus necessary for the proper preparation of solutions to be tested. This apparatus should include small percolators, drug mills, sieves, etc., for preparing tinctures or fluidextracts from Crude drugs, volumetric flasks, weighing bottles, pipettes, mortors and pestles, filtering apparatus, etc.

A very satisfactory location for the director's office is opposite the research laboratory. When so located it is oftimes possible, without inconvenience, for the director to step into the office and take care of incidental entry of records, dictation of letters, reports, etc., between observations of research experiments. Glass partitions as illustrated

make it possible to observe the activities of the laboratory from the director's desk. Desks, chairs, letter and index filing cabinets of mahogany or walnut finish with brown battleship linoleum floor covering make a fine contrast with the white enameled woodwork and walls.

All laboratory records such as duplicate reports, kymograph tracings, etc., may be concealed by placing them in transfer boxes

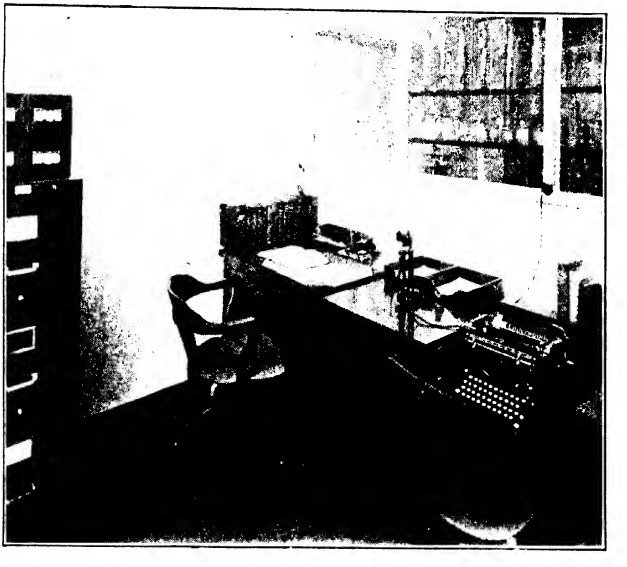

FIG. 102.1.—Director's office.

stored in cupboards of the type shown in Fig. 102*B*. Such a cupboard built across one side of the office finished in white enamel with brass hardware serves to conceal the files and blends nicely with the rest of the laboratories. The cupboard shown in the illustration is 7 feet high,—two sections high and two sections wide. Each section contains three shelves. If all of the shelves are not required for transfer cases, report books, etc., they may be used for filing

samples, storing preparations for deterioration tests, expensive reagents, etc.

Although the conditions as to available floor space and the products to be tested vary with each individual manufacturer, the above

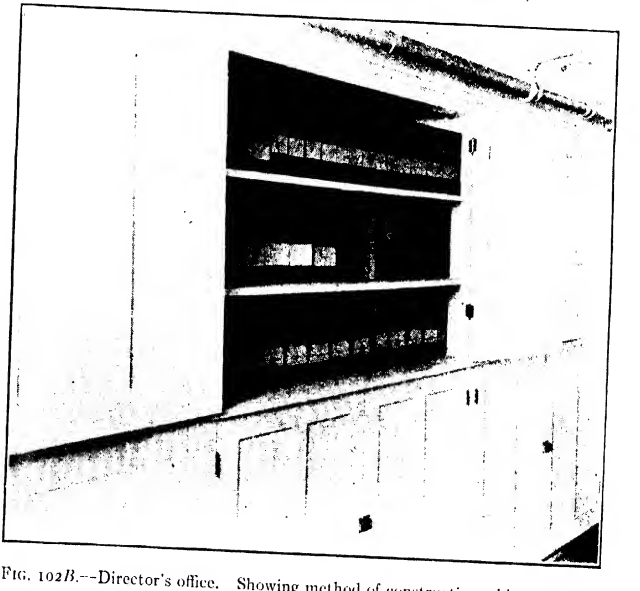

FIG. 102*B*.—Director's office. Showing method of constructing white enameled cupboards for filing reports, charts, etc.

short description covers the essential requirements of the biologic assay and pharmacologic research laboratories of the present day up-to-date pharmaceutical manufacturer.

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CHAPTER XIX

TECHNIQUE AND APPARATUS EMPLOYED

The purpose of this chapter is to give a description of the technique and apparatus employed in carrying out biologic assays. In order to familarize the student with all the more important apparatus used in experimental physiology, descriptions are also included of apparatus employed in demonstrating the *qualitative* physiologic action of drugs.

A large percentage of the apparatus used is that designed by Dr. William T. Porter and manufactured by the Harvard Apparatus Company. As I could not be expected to improve upon the descriptions of the designer I have in many cases quoted the descriptions of the apparatus devised by Dr. Porter from his "*Introduction to Physiology.*"

FIG. 103. All-glass syringe.

All-glass Syringe.—A syringe composed of two parts—*A*, plunger; *B*, graduated barrel. The sides of *A* are ground to fit accurately within the ground inner surface of *B*; the ends of *A* and *B* are also ground to fit into each other, thus ensuring complete emptying of the syringe and eliminating all possibility of leakage around the plunger. This syringe is particularly adapted for injecting small doses. It is especially valuable in intravenous work as it permits the operator to observe when all the air has been expelled from the syringe—a factor of the utmost importance in this class of work.

FIG. 104. Becker adjustable clamp.

Adjustable Clamp.—The adjustable clamp shown in Fig. 104 is compact and strong, made of bronze and nickel-plated. The screw clamps may hold a rod in vertical or horizontal positions. The movable half of the instrument can be swung through an arc of 140 deg. by turning a milled screw head.

Anesthetic Bottle and Air Warmer.—*For use in connection with the Respiration Pump.*

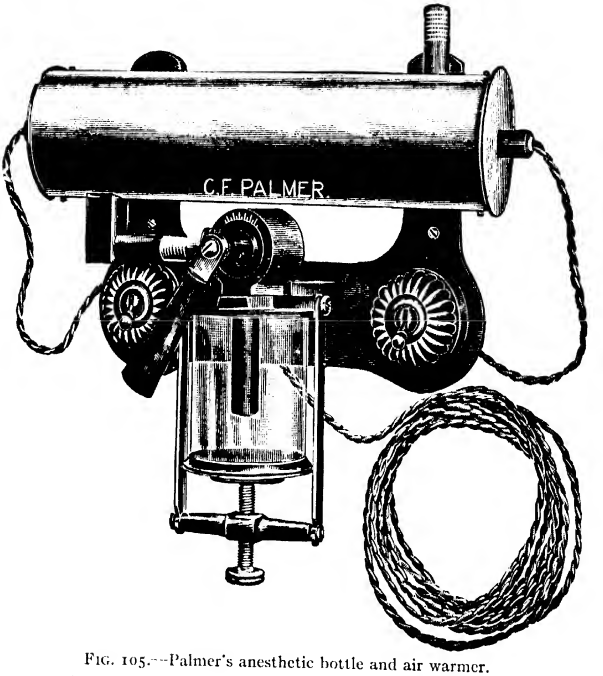

FIG. 105.—Palmer's anesthetic bottle and air warmer.

Referring to the above illustration, it will be seen that the bottle is similar to an ordinary specimen bottle with ground top, which is held up against a flat plate by a movable bridge piece and screw, it being only necessary to loosen the screw about two turns when the bottle can be removed for filling or cleaning. On the top of the plate is the regulating cock, which regulates the amount of air (and consequently

the strength of the anæsthetic) which passes through the bottle. Should the anæsthetic become used up during an experiment, it is only necessary to push the handle over till the pointer is at *O* of the graduated scale, when the bottle is entirely shut off, and can be removed for refilling: all the air passing direct to the heater, which consists of a brass tube with moveable ends, large enough to hold two ordinary electric lamps, there being two holders of the standard bayonet type provided, also two switches. It is advisable to have the lamps of different powers, say an 8 and a 16 C.P., then either the 8, the 16, or both, can be used according to the amount of air and degree of heat required. A little thick grease (as is the common practice in air pump experiments) may be smeared on the plate to make the joint round rim of bottle perfectly air tight. Depending from the plate into the bottle is a tube which causes the air to "blow" on to the surface of the anæsthetic, and as the latter becomes used up means are provided for lengthening this tube from outside the bottle.

Animal Holders. – In all experiments requiring operations upon anesthetized animals, the animals should, for convenience, be tied to a board.

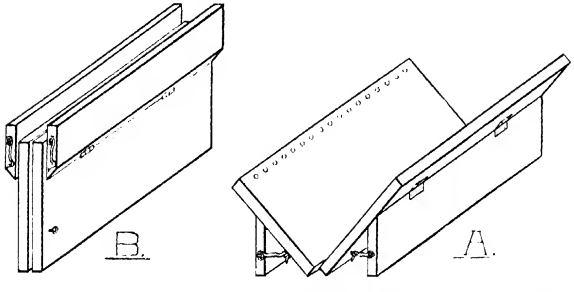

FIG. 106.—Simple, efficient and inexpensive folding animal holder, easily made from four pieces of board, six hinges and two hooks and eyes. *A*, open; *B*, folded.

(A) *Cats.*—It is best first to place cats in a metal etherizing box until under the influence of the anesthetic. They are then placed on a Harvard animal board, the head of the animal being held in position by means of a Czermak headholder.

(B) *Dogs.*—There are many complicated and expensive dog boards upon the market, but a simple V-shaped trough, with a series of holes along the upper edges through which the cords binding the dog are passed, answers all ordinary requirements. This series of holes extends the full length of the board thus making it possible to conveniently fasten any size dog to the board.

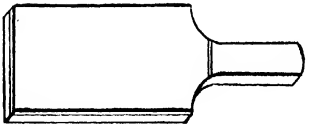

FIG. 107.—Frog board.

(C) *Frogs.*—The most convenient holder for frogs is a cork-covered board shaped as shown in the accompanying illustration. These boards should be about 5 in. × 7 in. with a 4-in. handle.

(D) *Rabbits.*—A small dog holder as shown above answers all ordinary requirements.

Artificial Respiration.—In intact animals pull the tongue forward to prevent any hindrance to the entrance of the air into the windpipe,

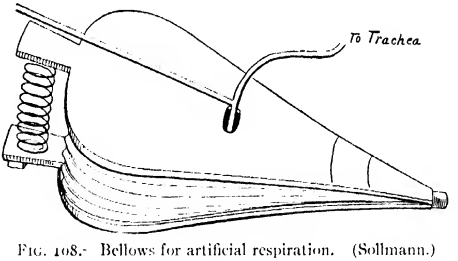

FIG. 108.—Bellows for artificial respiration. (Sollmann.)

then apply with the hands gentle intermittent pressure on the chest and abdomen. Care should be taken not to apply too much pressure as this is liable to rupture the lungs.

Artificial respiration is maintained during operations upon animals by means of some mechanical apparatus connected to the trachea by

means of a cannula. "The simplest device consists in a large bellows (15 by 22 in., exclusive of the handles). This may be arranged for foot power by fastening a spiral upholsterer's 'lounge spring No. 2' between the handles. The spout is closed with a cork. An inch hole is bored in the top. This bears a perforated cork, from which a tube leads to the tracheal cannula. A 'T' piece is inserted in the course of this tube, the free limb

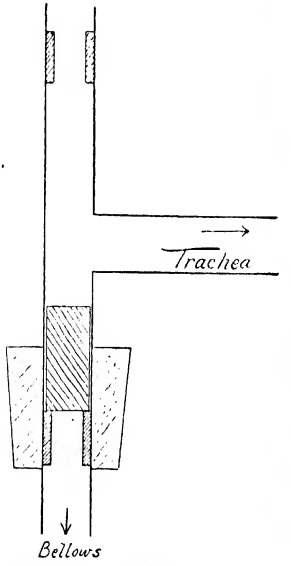

FIG. 109.—Hall's respiration valve. Natural size. (Sollmann.)

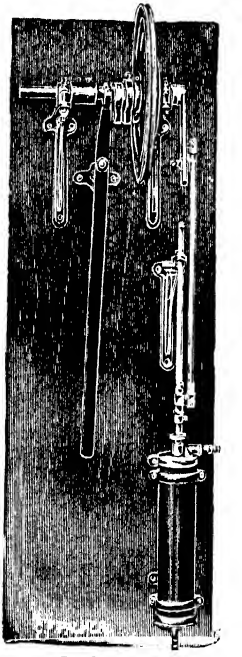

FIG. 110.—Dr. Broidie's respiration pump.

of the T being closed when the air is driven into the lungs, and opened when it is expelled. This may be done with the finger, but it is better to employ some automatic device. The 'T' piece may be replaced directly in the cork of the bellows. The free limb is connected with a rubber tube which is tied to the handle

in such a fashion that it is stepped on and closed when the bellows are compressed."[1] (Fig. 108.) (The spring may also be placed inside of the bellows.)

R. E. Hall has perfected a simple valve for this purpose. (Fig. 109.) It consists of a metal "T" piece with a steel plunger, well fitted and oiled, which is driven up by the bellows and falls back in expiration. The excursions are controlled by short pieces of rubber tubing inserted in the brass.

The number of respirations should be about 16 to 24 per minute.

There are many forms of mechanical apparatus on the market for maintaining artificial respiration during operative experiments. Two of the most efficient are shown in Figs. 110 and 111.

Artificial respiration and ether apparatus *for use with compressed air.* (Becker.)

This is an improved form of the apparatus designed by H. F. Pierce, A.B., B.Sc., (Oxon.) and described in the Journal of Laboratory and Clinical Medicine, December, 1923.

The apparatus will supply anesthesia, artificial respiration, and tracheal insufflation for any of the ordinary laboratory animals. It is light and compact and is easily carried about. The controls are located convenient to the hand, and ether, air, or respiratory rate may be varied and adjusted simply and easily.

In operation the apparatus is connected with a supply of compressed air at a pressure of 4 to 10 pounds per square inch. No other source of power is required.

Compressed air from the mains enters the apparatus at *A*. A part of the stream is shunted through the small rubber tube to the air motor.

Another part passes through the needle valve *B*, the interrupting cock *C*, and the ether apparatus, and out through *D* to the animal.

The handle *H* controls the proportion of air to ether in the mixture. When it is pushed to the extreme right, the ether is cut off, only air passes to the animal, and the ether bottle is tightly sealed so that it may be inverted without spilling the liquid.

The number of respirations per minute is controlled by the small knurled screw *E*.

[1] Sollmann's Manual of Pharmacology.

The amount of air passing to the animal is regulated by the needle valve *B*.

When artificial respiration is not required, the cuff *F* is revolved to show the full opening and the ether bottle then used in the ordinary way.

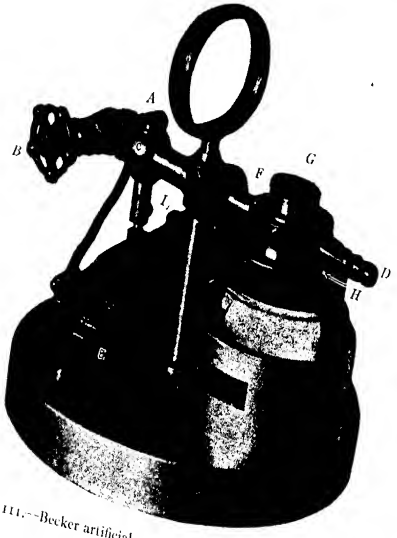

FIG. 111.—Becker artificial respiration and ether apparatus.

The ether may be replenished while the apparatus is in use by pushing the handle *H* as far as possible to the right, and removing the milled cap *G*.

By loosening the set-screw *I* and revolving the sleeve on the motor shaft, the period of the inspiratory phase may be lengthened or shortened.

By stopping the motor during the inspiratory phase of the cycle (by means of the screw *E*) a steady stream of air and ether vapor may be obtained for tracheal insufflation

Artificial Respiration Pump (*Dr. Brodie's*). The pump consists of a piston working in a barrel, 3 in. (76 mm.) in diameter and 11 in. (280 mm.) in length. By a simple adjustment of the crank the throw of the piston may be quickly altered to deliver any quantity up to one litre of air per thrust. The pump is driven by a 12-in. three-speed cone wheel, through a friction clutch actuated by a lever for stopping and starting. The valves are placed at the bottom of the cylinder and of the piston respectively. They are of simple con-

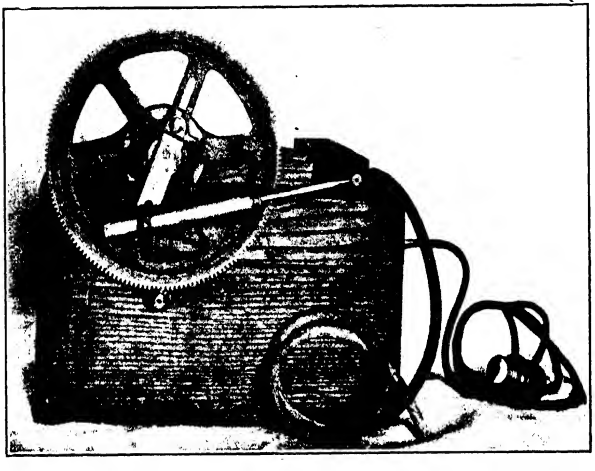

FIG. 112. Artificial respiration pump for small animals.

struction, and are easily reached for examination. The upper end of the cylinder is closed in and fitted with an intake tube so that any mixture of gases may be sent to the animal. The pump is mounted on a frame, so that it can be fixed in a vertical position on the wall of the experimental room. (See Fig. 110.)

A cheap and efficient artificial respiration apparatus for small animals such as guinea-pigs can easily be made by mounting a small bicycle hand-pump on a box; the outlet tubing is held stationary while the body of the pump is fastened to one spoke of a wheel near the rim. The wheel is rotated by means of a small motor, enclosed

in the box, thus working the pump. The amount of air expelled at each revolution of the wheel may be increased or decreased by regulating the drive of the piston with a set screw. (See Fig. 112.)

Batteries.—Either wet or dry cells may be used. The dry cells are the more convenient while the wet cells give a steadier current. The dry cells, however, suffice for most purposes.

(A) *Wet Cells, Daniell Cell.*—"The first constructed constant battery. It consists of a glass jar filled with concentrated solution of sulphate of copper, bathing an unclosed ring of sheet copper around a porous earthen jar filled with sulphuric acid (1 to 10 parts water) in which is immersed a rod of zinc. The zinc pole is the negative or cathode, and the copper pole the positive or the anode, and its electromotive force (E.M.F.) is about 1.07 volts."[1]

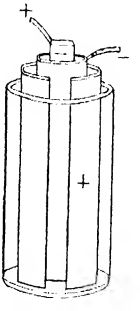

FIG. 113.—Daniell cell.

(B) *Dry Cells.*—The just described wet cell gives off fumes, contains acids, and must be prepared for use. As the dry cell is always ready and free from the preceding disadvantages it is usually preferred by laboratory workers. The dry cells are usually modified Leclanché batteries. The Leclanché cell consists of a glass jar containing a saturated solution of ammonium chloride into which an amalgamated zinc rod dips. The plate of carbon is fitted into a porous pot packed with pieces of carbon and manganese dioxide. The zinc is negative and the carbon positive. Its electromotive force is 1.5 volts. The dry cell is usually made of a zinc cup lined with plaster of Paris saturated with ammonium chloride. A carbon plate is placed in the center of this and surrounded with black manganese oxide.

Bistoury.—A narrow sharp-pointed surgical knife.

Bull-dog Clamps.—(Artery clips, Langenbeck's forceps.)

Burette.—An accurately graduated glass tube with a stopcock at one end. The burette is used for accurately measuring doses too large to be injected by an all-glass syringe. Also useful in making dilutions.

[1] Ott's Text-book of Physiology, 3rd edition, p. 575.

Colophonium Cement.—Is used for fastening writing points to heart, muscle, respiration levers, etc. This cement is a mixture of colophonium resin and bees wax, which softens at a very low temperature and quickly hardens.

Cannulæ.—(*a*) *Vessel cannulæ* are short tubes of the shapes shown in the following illustrations used for connecting blood-vessels with various apparatus. The small end is tied in the vessel while the larger end is connected with a tube leading either to a recording apparatus or from a burette used for measuring doses.

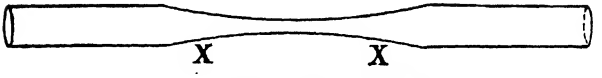

FIG. 114.—Tube drawn for making cannula.

German silver cannulæ may be purchased from various manufacturers of physiologic apparatus but with a little practice one may easily make his own from glass. First heat the proper size tubing in a blow pipe flame and draw it out in the form of Fig. 114; cool, heat at point *X* with a *sharp-pointed* flame and again draw gently to the form of Fig. 115; cool, file and break off at 1; put on grindstone and grind off tip to dotted line, round off edges in flame.

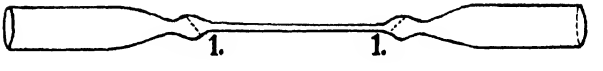

FIG. 115.—Second step in making glass vessel cannula.

Cannulæ should be made in various sizes in order to fit the vessels of different animals.

The most important point in glass blowing is to heat evenly the entire circumference of the tubing at the point where it is to be drawn.

One objection to the straight cannulæ shown above is the frequency with which they become clotted. I have found that by using a cannula of the style shown in Fig. 117, this objectionable clotting may be almost entirely eliminated. The superiority of this cannula is due to the fact that the bulb contains a comparatively large

amount of magnesium sulphate or sodium citrate solution, thus giving the blood a greater quantity of liquid with which to diffuse, and thereby increasing the length of time required to produce clotting.

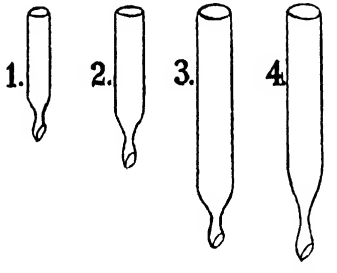

FIG. 116.—Cannulæ for vessels. 1, For guinea-pigs; 2, for rabbits and cats; 3, for medium size dogs; 4, for large dogs.

To make a cannula of this type, first, seal one end of a 5-in. piece of glass tubing; heat the central portion to a white heat and blow a bulb about 2/3 in. in diameter; then finish as described on page 300.

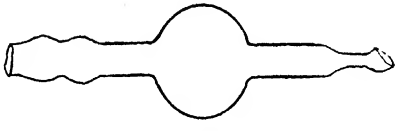

FIG. 117. —Improved vessel cannula.

(*b*) *Tracheal Cannulæ.*—There are two styles on the market, one of German silver and the other of glass.

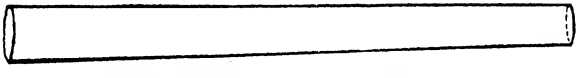

FIG. 118.—Tube drawn for making tracheal cannula.

Tracheal cannulæ can be made from glass as follows: Take a piece of glass tubing about 5 in. long, evenly heat one end in a "fish tail" flame and draw out slightly so that the one end is somewhat smaller than the other. (See Fig. 118.) This will make it possible

to use the cannula for different sized trachea. The two ends should now be ridged to facilitate tying it into the trachea. This is done by evenly heating a small band of the tubing and while hot pushing the ends together. (See Fig. 119.) The tubing is then file-marked, broken off at 1 and 2, and the ends rounded in the flame. The tube is then evenly heated in the middle and bent at right angles.

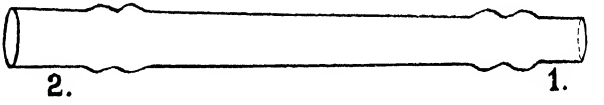

FIG. 119.—Second step in making glass tracheal cannula.

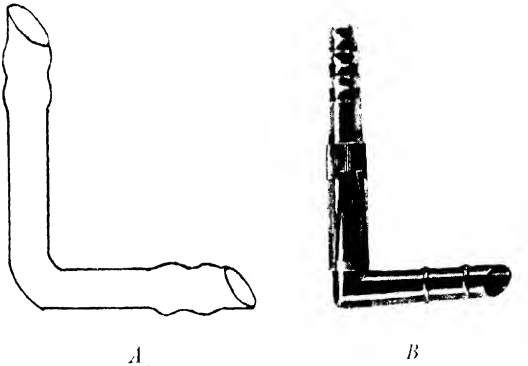

FIG. 120.—Tracheal cannulæ; *A*, glass, *B*, metal. (Becker.)

Charts. (*a*) *Method of Smoking.*—Charts are best smoked with a luminous gas flame. A burner well adapted to this purpose (see Fig. 121) can easily be made as follows. Take a piece of 1/2-in. gas pipe about 12 in. long, and close one end with a cap; bore holes about 1/16 in. in diameter every 1/4 in. from the capped end to within about 1 1/4 in. of the other end. The uncapped end is then attached to a rubber gas tube. In order to produce heavy black smoke the illuminating gas should be passed through a wash bottle containing benzol. In the absence of gas, charts may be smoked with an oil

lamp provided with a wide wick. Heavy oils should be used in order to produce the maximum amount of smoke.

To Smoke Single Drums.—First cover drum with glazed paper and then place upon the sleeve. The sleeve is then twirled between the fingers while holding the drum directly above the flame as shown in Fig. 121.

FIG. 121.—Method of smoking single drum.

To Smoke Double Drums.—To smoke paper for a *Harvard double Drum Kymograph* remove clock works, then place the frame on the Harvard smoking stand and insert the turning crank at the end of the drum rod. Put the long band of paper over the drums and tighten it by moving the rear drum. Revolve the drum by means of a turning crank while holding the gas flame beneath the paper band at such a height that the paper shall pass just below the free edge of the flame. In order to prevent the paper from crawling when the drums are revolved it is necessary to exercise great care in overlapping the edges of the paper where it is gummed. In pasting the

ends of the paper together it is best first to lay the paper full length on the table; both ends are then lifted and brought toward each other until they overlap about 1 in. *At this point the operator should see that the edge of the upper layer of the paper coincides throughout its*

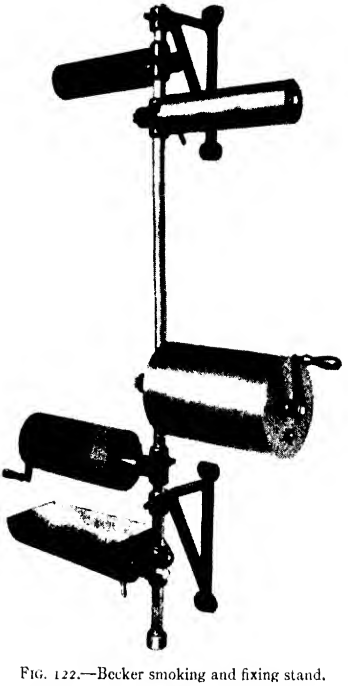

FIG. 122.—Becker smoking and fixing stand.

entire length with the edge of the lower paper. While holding in this position the over-lapping edges of the paper are pasted together.

To smoke paper for large kymographs of the Becker type (Fig. 139 p. 327) a special smoker is required. The smoker shown in Fig. 122 is specially designed for smoking and fixing papers up to fifteen feet in length. The supporting rod is held to the wall by

means of two large brackets. The smoking drums are of brass while the fixing drums are made of wood. The drums may be secured in any position on the supporting rod. The lower fixing drum revolves inside of the trough which holds the fixing solution. An outlet for emptying the solution is conveniently placed in the bottom of the trough.

(*b*) *Method of Fixing Charts.*—Charts may be rendered permanent or "fixed" by passing them through an alcoholic solution of benzoin

FIG. 123.—Typography of dog's neck: *a*, sternohyoid muscle; *b*, vagus; *c*, carotid artery; *d*, trachea. The carotid artery and vagus have been removed from the sheath and drawn apart.

or varnish. The strength of this solution depends upon the operator's method of filing his charts. If they are small and do not require folding the solution should be rather concentrated, which will give a fine glossy appearance. If the charts are large and require folding the solution should be rather dilute (just strong enough to prevent the carbon from rubbing off)—concentrated solutions cause the charts to crack when folded, and for this same reason dilute varnish is to be preferred to benzoin.

If the tracings are to be used for making half-tones or zinc etchings for reproduction in journals, etc., the two following precautions should also be taken: (1) Paper should be smoked as black as possible; (2) fixing solutions should be filtered and be as dilute as possible, as concentrated solutions cause the tracings to turn yellow thereby greatly increasing the difficulty of making the reproductions.

Charts which have become disfigured by use, or scratched, may be retouched before making reproductions by painting over the disfigured places with paint made from lamp-black and turpentine. After this, the charts can be re-fixed. This simple procedure will in most cases greatly improve the appearance of the reproduction.

Carotid Artery, Method of Exposing.—After clipping the hair from the neck a long incision is made in the median line from the thyroid to the breastbone. The edges are then held apart and the position of the sternomastoid and sternohyoid muscles determined. Following the edges of the sterno-mastoid the incision is carried into the groove and the attachments exposed. The carotid artery is at once detected by its strong pulsations. After roughly cleaning, it is lifted to the surface by means of a tenaculum or the fingers. In the dog the vagus, sympathetic and depressor fibers lie in the same sheath as the carotid artery. In the rabbit these run separately but may be recognized by their size—the vagus being the largest, the depressor the smallest. The carotid is then carefully and gently separated from the vagus. See Fig. 123.

For method of connecting artery with manometer, see page 66.

Chronograph.—See signal magnet, Figs. 145 and 146, page 334.

Clotting.—In blood-pressure experiments if the writing point of the manometer ceases to pulsate while the heart beat can still be felt, clotting has occurred, either in the cannula or the connecting tube. In such cases the artery and connecting tube should be clamped and disconnected. Both should then be cleaned and reconnected. Upon releasing the clamps the writing point should again record the pulsations of the heart.

Constant Temperature.—For methods of maintaining constant temperature see Chapter XIV, p. 199.

Defibrinated Blood.—This is prepared by first whipping the blood, and then straining it through cloth.

Electrically Heated Operating Table. This new table, designed by Dr. Brodie, is larger than the old ones, having a top 51 in. (129.5 cm.) × 18 in. (46 cm.) × 40 in. (101.5 cm.) high. It is fitted all round with cleats into which the holding down cords can be easily and quickly fixed. Near the center and flush with the top is a copper hot plate, 30 in. (76 cm.) × 12 in. (30.5 cm.), heated by means of

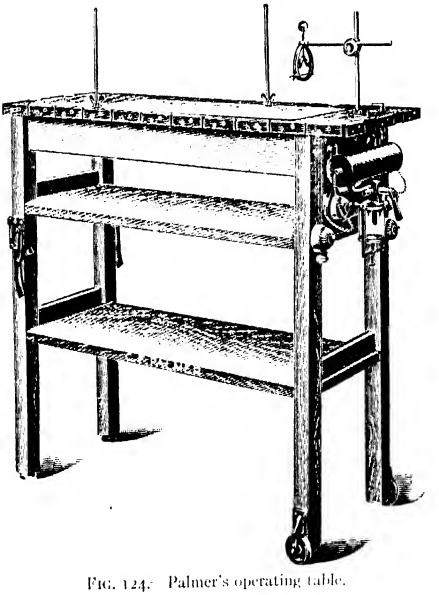

FIG. 124.—Palmer's operating table.

two electric lamps, each having its own independent switch. Two upright rods, working in slots, are also provided, and will be found useful for many purposes. Dr. Brodie's well-known anesthetic bottle and air warmer (see Fig. 105) is now fitted to these tables with a bent tube projecting through the top to supply the air to the animal. The table is fitted complete with animal holder, four controlling switches, main switch and plug; also wheels and handles for convenience in moving.

Electrodes.—From his instruction on the theory of electrolytic dissociation the student should be familiar with the fact that when metal electrodes come in contact with an electrolyte in solution, polarization currents develop. Ordinary metal electrodes in contact with a muscle or nerve will be surrounded by lymph, and in this fluid electrolysis will take place during the passage of an electric current. The ions resulting from this electrolysis will be positive and negative respectively; if, therefore, the circuit of this seat of chemical and electrical change is suddenly made or broken, a shock will be produced, for the wires of electrodes surrounded by the electrolyzed fluid will form a minute battery. This can be demonstrated by the following experiment: A pair of electrodes connected with DuBois key is placed under the sciatic nerve, which has been exposed in the thigh of the pithed frog. Making or breaking the circuit causes no contraction. The two wires of a Daniell cell are connected with each side of the DuBois key and the current is allowed to pass through the nerve for several seconds. Then these two wires are rapidly disconnected from the battery and key; the key is closed and opened, and each time a contraction of the muscles of the leg is caused. This make and break can be repeated several times with a similar result, until the polarization has disappeared.

This experiment shows the necessity of employing *unpolarizable electrodes* in experiments upon the effects produced in nerve and muscle by the passage of a constant electric current. Electrodes made of metal must for this reason be avoided. Strictly speaking no electrode is nonpolarizable, but practically all the polarization errors are excluded in the "boot electrode." These electrodes are boot-shaped, made of Potter's clay, and were designed by Prof. W. T. Porter.[1] The leg is pierced with a hole 28 mm. deep and 8 mm. in diameter, in which is placed the zinc. The foot is twenty millimeters long measured from its junction with the leg. In the foot is a well for normal saline solution which shall keep the feet equally saturated. In use the hollow leg of the boot is half-filled with saturated solution of zinc sulphate and the rods of amalgamated zinc are immersed in the latter.

[1] W. T. Porter: "Science," 1901, XIV, p. 567–570. The well was added in Nov., 1905.

The boot electrodes when used are mounted in rubber holders in the apparatus described as the "moist chamber." (See Fig. 142.)

Frogs. *Method of Weighing.*—Frogs can be easily and rapidly weighed by first counter-balancing a frog cage after which the frog to be weighed is placed within the cage, the lid replaced, and the weight determined in the usual manner.

Frog Cages.—Immediately preceding and during test periods frogs should be kept in cages divided into compartments for each individual frog. See Fig. 83 p. 203.

Guinea-pig Boxes.—Guinea-pigs are best kept during test periods in small galvanized iron boxes about 9 × 16 × 7 in., with lid containing a window covered with wire netting of about 1/4-in. mesh. These boxes accommodate four pigs each. If the boxes are numbered and four pigs all of different colors or markings are placed in each box, the pig which has received any given injection can be distinguished without the objectionable use of tags, labels, etc.

Small stock cages of the type shown in Fig. 86 p. 232 are also very convenient for this purpose.

Head Holder.—A head holder is not generally needed in biologic standardization work. It is, however, advisable to use a head holder of some sort when working with cats.

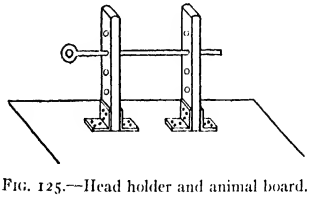

FIG. 125.—Head holder and animal board.

A holder of the design shown in Fig. 125 is cheap, easily made, and answers almost every purpose. The neck is placed between the two vertical posts and the bar placed across it. This holder surrounds the neck without compression and the head cannot be withdrawn. When using dogs the cross piece is pushed back of the teeth; a piece of stout twine is then passed under the neck, behind the ears, the ends are brought forward, wound tightly around the cross piece and

tied about the mouth. A more expensive and efficient holder is shown in Fig. 126.

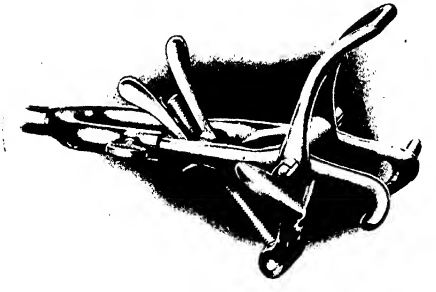

FIG. 126. The Czermak head holder. About one-half actual size.

Heart Lever.—This very light lever is used in the suspension method of recording the contractions of the heart, or for similar purposes. The axle is 7 mm. in length. The axle, with its aluminum wire, 22 cm. long, weighs about 0.4 gm.

FIG. 127.—Hitchens syringe.

Hitchens Syringe.—The Hitchens syringe is composed of three parts, *i.e.*, (1) glass body of syringe; (2) needle, and (3) rubber bulb. This syringe is especially designed for administering subcutaneous

injections. It is particularly adapted to physiologic standardization work as it allows no possibility of loss while inserting the needle and may be washed with water without being withdrawn. For detailed method of its use see page 55.

Inductorium.—The following illustration shows the Harvard induction apparatus.[1] The primary coil wound with double silk-covered wire of 0.82 mm. diameter, having a resistance of 0.5 ohms, is supported in a head piece bearing three binding posts and an automatic interrupter. The core consists of about ninety pieces of shellaced soft iron wire. This core actuates the automatic inter-

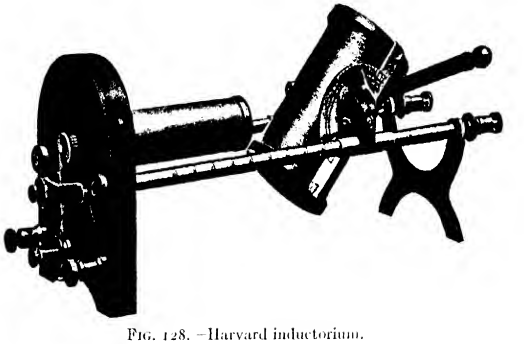

FIG. 128.—Harvard inductorium.

rupter. The interrupter spring ends below in a collar with a screw. By loosening the screw, the interrupter with its armature may be moved nearer to or farther from the magnetic core. Once set, the interrupter will begin to vibrate as soon as the primary circuit is made. The outer binding posts are used for the tetanizing current. The left-hand outer post and the middle post are used when single induction currents are desired; they connect directly with the end of the primary wire thus excluding the interrupter. These several connections are all in view; there are no concealed wires.

From the head piece extend two parallel rods 22 cm. in length, between which slides the *secondary coil*, containing 5000 turns of

[1] W. T. Porter: American Journal of Physiology, 1903, p. 35.

silk-covered wire 0.2 mm. in diameter. Over each layer of wire upon the secondary spool is placed a sheet of insulating paper. Each end of the secondary wire is fastened to a brass bar screwed to the ends of the hard rubber spool.

The brass bars bear a trunnion which revolves in a split brass block, the friction of which is regulated by a screw. The trunnion block is cast in one piece with a tube 3 cm. in length, which slides upon the side rods. A set screw, not shown in Fig. 75, holds the trunnion block tube and the secondary spool at any desired point upon the side rods. This screw also serves to make the electrical contact between the trunnion block tube and the side rod more nearly perfect. The secondary spool revolves between the side rods in a vertical plane. When the secondary coil has revolved through 90°, a pin upon the side bar of the secondary coil strikes against the trunnion block and prevents further movement in that direction. The right-hand side bar bears a half circle graduated upon one side from 0° to 90°. An index-pointer is fastened upon the trunnion block. One side rod is graduated in centimeters.

The side rods end in the secondary binding posts, so that moving the secondary coil does not drag the electrodes. Next to the binding posts is placed a substantial "knife-switch" short-circuiting key, with hard rubber handle.

Injections. A. Subcutaneous.—Are injections made under the skin.

1. *Mammals.*—Subcutaneous injections are best made in mammals by means of a Hitchens syringe. In most cases the abdominal site is to be preferred (for detailed description of method of employing the Hitchens syringe see page 55). By this method absorption occurs more rapidly than when the drugs are given by mouth, the local action on the alimentary canal is avoided, and the operator is more certain to get the full effect of the remedy, provided it is soluble and is not precipitated at the point of injection. Care should be taken not to pierce blood-vessels, veins or the abdomen with the needle. Irritant drugs should not be injected by this method as they cause great pain, swelling and sometimes suppuration, even when the injection has been carried out aseptically. As the absorption from the subcutaneous tissues is so much more rapid than that

of the stomach and intestine, the dose required to produce a given effect by this method is necessarily much smaller than when given by mouth.

2. *Cold-blooded Animals* (frogs). Due to the lack of elasticity of the skin of this class of animals the perforation in the skin fails to close upon withdrawal of the needle. It is advisable, therefore, first to pass the needle through muscular tissue in order to prevent the possible loss of part of the injected fluid. In frogs this can be accomplished by passing the needle through the muscle of the leg into the anterior lymph-sac or, better still, force open the

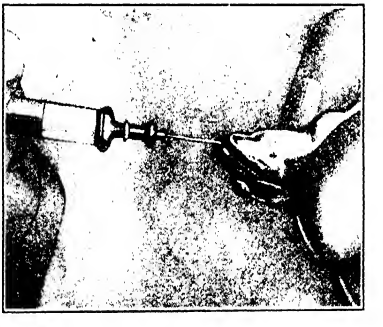

FIG. 129.—Method of injecting frogs.

mouth, pass the needle through the lower wall into the anterior lymph-sac. By this latter method any fluid which might possibly escape would merely pass into the mouth and be absorbed there. (See Fig. 129.)

B. **Intravenous injections** are injections made into the vein. This is the most certain method of bringing drugs into the circulation and tissues, and is at the same time the most rapid. This method is therefore very largely used in experiments upon animals.

1. *Operated Animals.*—In dogs, cats, rabbits, etc., intravenous injections can be made in either jugular or saphenous vein. I prefer the latter, however, because being situated at greater distance

from the heart it gives the preparation injected opportunity to diffuse more thoroughly with the blood before it reaches the heart.

Method of Injecting.—The saphenous vein is lifted and held with a pair of tweezers while the needle of the all-glass syringe is inserted far enough through the cannula into the saphenous vein to allow the point to project into the femoral vein (Fig. 130). After injecting the preparation withdraw the needle and quickly clamp saphenous vein with a bulldog clamp.

The advantage of this method is that although the clamping off of the saphenous vein after withdrawing the needle causes clotting,

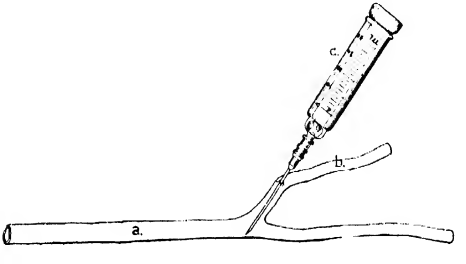

FIG. 130.—Method of injecting. *a*, Femoral vein; *b*, saphenous vein; *c*, all-glass syringe.

the preparation injected is carried to the heart by means of the main current of blood in the femoral vein.

2. *Intact Animals.* (*a*) *Dogs and Cats.*—A ligature is tightly tied above the second joint of one of the hind legs; after the veins have dilated sufficiently to show their location the hair is clipped or shaven from over the internal metatarsal vein; the needle is then inserted, the ligature removed and the drug injected.

A very practical method of making intravenous injections especially for inexperienced persons is given by J. J. Watson[1] which is especially useful in work upon dogs. The limb is corded above the joint so as to cause the vein to become prominent. The vein is then transfixed with an ordinary sewing needle. The cord may then be

[1] Watson: Jour. Amer. Med. Assoc., Vol. LXII, No. 3, p. 383.

loosened and the needle of the syringe inserted into the vein at right angles and beneath the sewing needle which is raised by a hemostatic forceps. The accompanying drawing illustrates the advantages of this method.

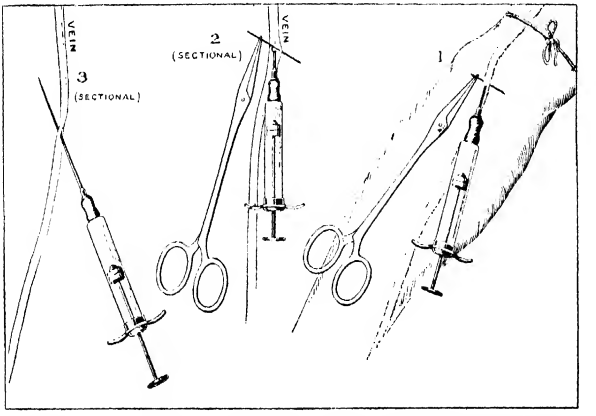

FIG. 131.—No. 1 shows the method of transfixing and raising the vein with a sewing-needle and holding it in the elevated position by means of a hemostat. The syringe needle is shown inserted into the vein beneath the transfixing needle. No. 2 shows more in detail the method of fixation and the insertion of the needle. No. 3 shows what frequently happens in attempting to insert the needle of the syringe without first fixing the vein.

(*b*) *Rabbits.*—Intravenous injections are most conveniently made in the ear of these animals. The translucent structure of the ear enables the operator on holding it in a vertical position between himself and the source of light to see the exact location of the veins. It is then comparatively easy to insert a fine needle and inject the drug. The rabbits ear should be washed with soap and water, shaved and then painted with xylol which distends the veins and thus greatly facilitates the insertion of the syringe needle. Upon withdrawal of the needle the puncture should be painted with flexible collodion and covered with a small piece of absorbent cotton. If this fails to stop the flow of blood a small paper clip should be fastened to the ear directly over the puncture.

Figure 132 shows a convenient method of holding rabbits while giving intravenous injections. The box is 20″ long, 6″ wide and 7″ high. The lid is hinged on one side and is supplied with a hasp and staple for fastening the other side. The front end is divided into two halves. The bottom half is stationary while the top half is arranged to slide in a groove. A small bolt attached to the lid is so arranged that it may be inserted into holes in the back of the top half of the front and thus hold this half in any desired position. The back of the box is arranged to slide in a groove so that the space

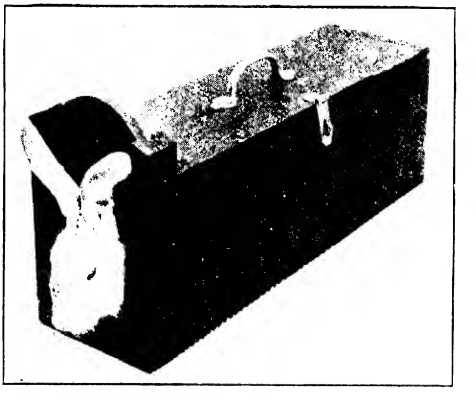

FIG. 132.—Box arranged for holding rabbits while administering intravenous injections.

within the box may be lengthened or shortened to suit the size of the rabbit. The sliding back is held in position by a small bolt which fits into any of a series of holes in the bottom of the box.

(*c*) *Mice.*—In these animals intravenous injections are best made in the tail by means of a fine needle.

C. *Intramuscular injections* are made directly into the muscles by deep vertical punctures. An ordinary hypodermic needle generally answers the purpose. Care should be taken not to enter any of the viscera, vessels or nerve sheaths.

Insertion of Cannulæ into Vessels.—Cannulæ may be easily inserted into vessels by the aid of a small tenaculum. The pointed

end of the tenaculum is first slipped into the vessel through a small V-shaped aperture previously made for the purpose. The aperture is held open by lifting the tenaculum (Fig. 133) and the nozzle of the cannula gradually slipped in. As the cannula is being inserted the tenaculum is gradually withdrawn and the artery then tied fast by means of a ligature.

FIG. 133.—Method of inserting cannula into vessel. *a*, Tenaculum; *b*, carotid artery; *c*, cannula; *d*, connecting tube.

Isolated Mammalian Heart. *Apparatus Necessary for the Study of Same.*—The mammalian heart isolated completely from the body can be maintained in constant activity for several hours by perfusing with oxygenated Loche's solution, containing a small quantity of defibrinated blood, at body temperature (38° C.) and under a pressure of about 130 cm. of water, which permits the study of its functions under simple conditions. By adding drug solutions to the perfusion liquid their action may be readily recorded upon the

kymograph. The hearts of cats and rabbits are especially well adapted to this experiment; they are preferable to the dog's heart on account of their smaller size.

Preliminary Operations.—As soon as the apparatus has been assembled (see Fig. 134) and ready for use the animal is anesthetized and the carotid artery and femoral vein exposed. The animal is then bled from the artery. As soon as the flow ceases the artery is clamped. The blood is then defibrinated, heated to about 45° C. and poured into the perfusion bottle. The clamp is then removed from the carotid artery and the animal again bled. The maximum amount of blood is obtained by allowing Loche's solution to flow into the femoral vein from a burette. This mixture of blood and Loche's solution is then defibrinated, strained, mixed with the blood first drawn, again heated to 45° C. and replaced in the perfusion bottle. The heart is then quickly excised and tied to the cannula connected with the apparatus; care being taken that the cannula does not interfere with the play of the semilunar valves. The apex of the heart may then be connected with the writing lever by means of a hook-shaped pin and silk thread, or a Guthrie cardiograph may be employed.

When the perfusion is started the pressure closes the semilunar valves, so that the fluid is forced through the coronary circulation, escaping through the right auricle. The solution may be collected in a beaker and the undrugged portions returned to the perfusion bottle and used again. The flow should be rather free. After a few minutes the heart commences to beat feebly and irregularly but soon develops strong regular contractions.

After recording a normal tracing the effects of a drug may be demonstrated by replacing the plain Loche's solution by the medicated solution. After recording the effects of the drug, the heart is again perfused with unmedicated Loche's-blood solution, which causes the heart quickly to return to normal, after which another experiment may be performed.

Apparatus.[1]—"The points to be secured in the isolated heart apparatus are: 1. A uniform temperature of about 37° Centigrade. 2. An adjustable pressure for the perfusion fluid. 3. A device for

[1] Greene's Experimental Pharmacology, 1909, p. 73.

quickly shifting from the normal perfusion to the drugged perfusion fluid without change in temperature, pressure, or any other factor than the presence of the drug. 4. An accurate recording device.

"The apparatus shown assembled in Fig. 134 accomplishes all of the above points. The gas water heater connected as shown will

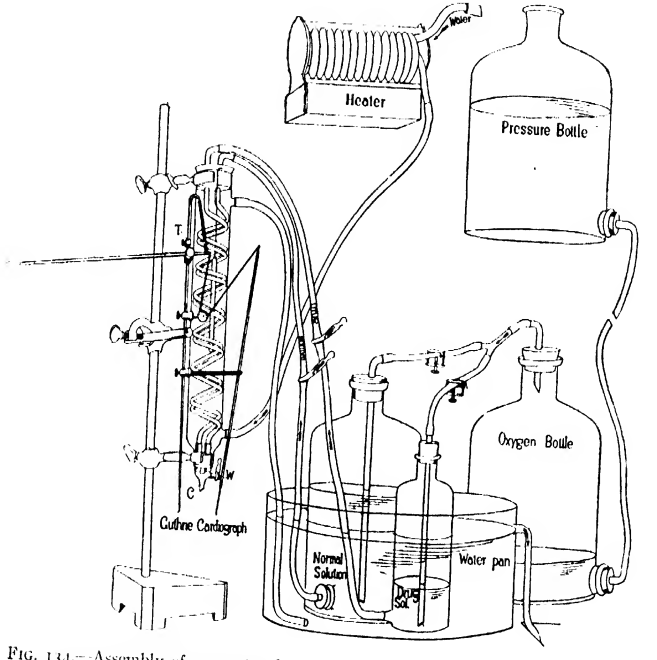

FIG. 134.—Assembly of apparatus for the pharmacologic study of the isolated heart of a mammal. (Greene's Experimental Pharmacology.)

maintain a uniform temperature in the water jacket through which the perfusion tubes run to the heart cannula. The overflow from the water jacket is conducted into a pan in which the perfusion fluid reservoirs receive preliminary warming. The heart is attached to a very short cannula beneath the warming jacket and the overflow of

perfusion fluid maintains a temperature of the heart only slightly below that of the warming jacket.

"The pressure on the heart, *i.e.*, on the perfusion fluid, is accomplished by connecting the perfusion bottle with an air or oxygen reservoir, and this in turn with a water reservoir which can be raised or lowered. The flow of water from the pressure bottle into the closed system produces the desired pressure on the perfusion system. At the same time the perfusion fluids are aërated by the air (or oxygen) as it is forced into the reservoir, a result accomplished by conducting the perfusion bottle inlet tubes to the bottom of the containers.

"A uniform pressure is secured on both the normal and the drugged perfusion fluids by the system of tubes shown. If the clamp is removed from the outflow tube of the drugged perfusion fluid at the exact moment a second clamp is placed on the tube from the normal fluid reservoir (or *vice versa*), the shift will be accomplished without change of pressure on the heart. The tubes run independently to the cannula which is itself so short that the time from the moment of turning a perfusion fluid on or off is reduced to a minimum. The cannula is provided with a side washout tube.

"The Guthrie cardiograph shown is very adjustable in all essential features. It gives satisfactory and accurate records, if care is used in inserting the lever tips into the walls of the heart. This apparatus permits a direct record on the ordinary kymograph. It also permits one to surround the heart with a warm cup or jacket where greater constancy of temperature is desired, as in research work."

The apparatus described above answers all ordinary requirements, but, for work requiring the greatest degree of accuracy, constancy of temperature, etc., the best apparatus is perhaps the one devised by Eyster and Loevenhart.[1] Their description of the apparatus follows:

"In the study of the action of substances upon the isolated mammalian heart it is of prime importance to remove all variations of temperature and pressure in order to be sure that small changes in rate, amplitude or tone are really due to the action of the drug on the heart and not simple temperature and pressure effects incident to

[1] Eyster and Loevenhart: Journ. of Pharmacology and Experimental Therapeutics, Sept., 1913, p. 57.

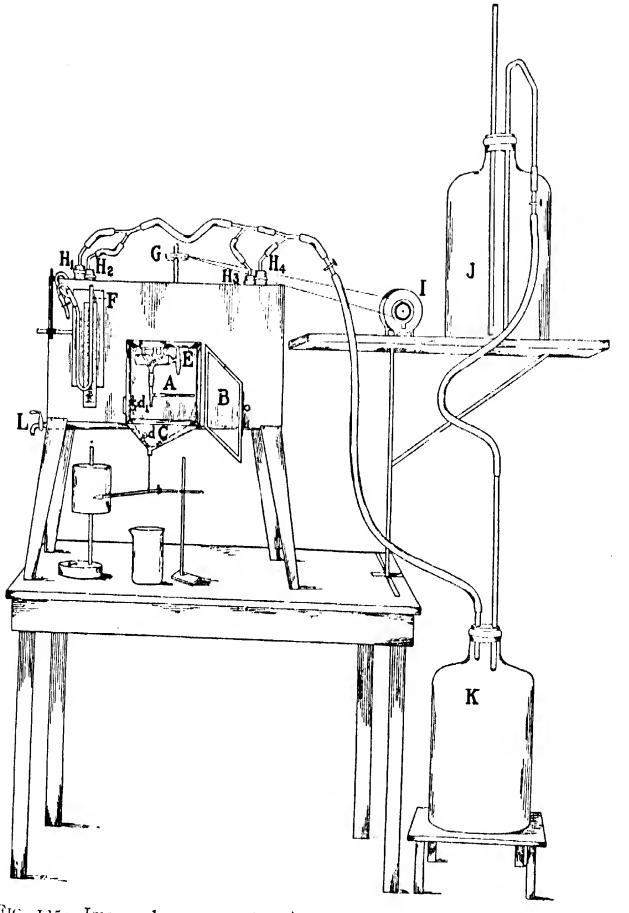

FIG. 135.—Improved apparatus for the pharmacologic study of the isolated mammalian heart. (Side view.) (Eyster and Lovenhart.)

changing from one solution to another. In all forms of perfusion apparatus in which the perfusion fluid is heated by passing through a

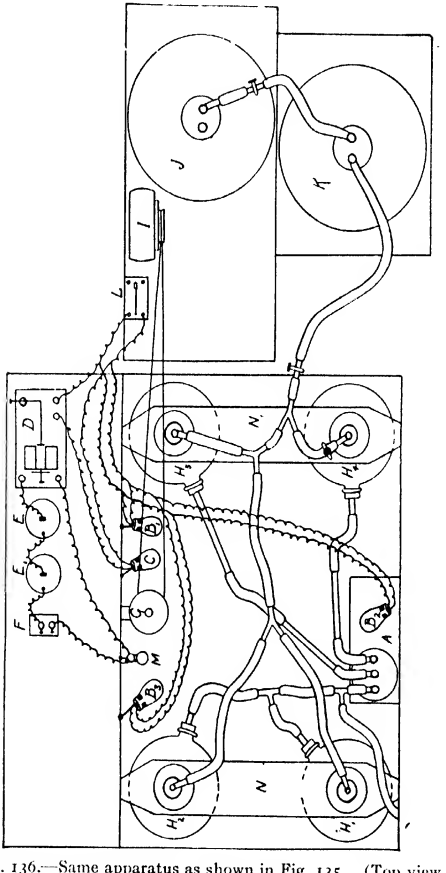

FIG. 136.—Same apparatus as shown in Fig. 135. (Top view.) (Eyster and Lovenhart.)

metallic coil placed in hot water, the temperature which the perfusion fluid assumes depends on the rate of flow through the coil. When

the outflow becomes slower the perfusion fluid becomes warmer due to the delay in passing through the coil and when the outflow is increased the temperature of the perfusion fluid falls. Furthermore, the change from one perfusion fluid to another should be made quickly and all avoidable dead space should be eliminated.

These are the points which we had in mind in designing the apparatus shown in Figs. 135, 136, and 137.

The tank is of copper, lined with tin. It is 29 1/2 in. long, 18 in. wide and 15 in. deep. The chamber A in which the heart is suspended is 8 3/4 in. wide, 8 in. high and 4 in. deep. This chamber is surrounded on four sides by the water of the tank at the temperature of the perfusion fluid. The glass door *B* in front is on a hinge and the heart can be clearly seen and is readily accessible. The floor of the chamber is funnel shaped so that the perfused fluid may be collected, measured, and analysed if desired, or in case it is desired the fluid may be returned to the bottle and perfused again. Through the opening the thread passes from the heart to the lever. By means of the pulleys *d* and d_1, the auricular beat may be recorded simultaneously with that of the ventricles. The various solutions to be studied are placed in the four bottles H_1, H_2, H_3, and H_4 in the tank and allowed to come to the temperature of the tank. All of the bottles are under the same head of pressure. The bottles are provided with an opening near the bottom (aspirator bottles) which is closed by a rubber stopper. A glass tube passed through the stopper is connected with the four-way stop-cock by means of rubber tubes. Bottles H_1 and H_2 are usually kept for the control solution and are so arranged that either may be connected with the left-hand tube of the four-way stopcock. The manometer *F* is connected with the tube from bottle *H*, and gives the pressure of the perfusion. The glass stopcock is made so that there is no constriction in the stopcock. The bore through the stopcock is 4 mm. and the openings are so placed that we can change from any one of the three solutions to any other one directly. By this means the dead space is reduced to a minimum. As the bottles become empty they are prevented from floating by means of the metal sheets *N* and N_1, shown in Fig. 136. These sheets fit on a runner at either side and are provided with set screws.

Very active stirring is maintained by means of the fan *G* connected with the motor *I*, by means of a belt. The tank is further provided with a sheet iron bottom which is made in one piece with the supporting legs. This gives greater stability to the apparatus and protects the bottom in case Bunsen burners are used to raise the temperature of the tank quickly to that desired.

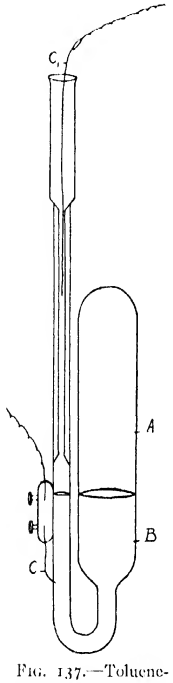

FIG. 137.—Toluene-mercury regulator used to automatically control the temperature of apparatus shown in Figs. 135 and 136. (Eyster and Lovenhart.)

The bottom of the tank is 19 1/2 in. above the table so that a kymograph and other recording apparatus may be placed under it. The faucet *L* is useful in emptying and cleaning the tank. The tank is heated by electric-light bulbs. Three 32-candle-power and one 16-candle-power bulbs may be used according to the temperature of the room and that required for the perfusion. One of these bulbs, preferably the 16-candle-power bulb, is connected with a relay through which the current is made and broken by means of a toluene mercury regulator, Fig. 137. The portion *A* of the regulator is filled with toluene and the portion *B* with mercury. The relay is operated by two dry cells. By this arrangement the temperature of the tank can readily be kept practically constant, the variation being from 0.01° to 0.02° C. Since the entire mass of fluid is immersed in the water of the tank the temperature of the perfused fluid does not alter regardless of the rate of perfusion.

Jugular Vein. *Method of Exposing.*—The external jugular vein may be reached through the same incision made to expose the carotid artery; it is exposed by blunt dissection between the sternomastoid muscle and the skin. It may also be reached directly by a skin incision made about the middle of the neck, in a line drawn from the angle of the jaw to the manubrium.

Kymograph.[1]—"The improved kymograph consists of a drum revolved by clockwork and also arranged to be more rapidly revolved or "spun" by hand.

The drum is aluminum, cast in one piece turned true in the lathe to a circumference of 50 cm. The height is 15.5 cm. and the weight is about 600 gm. The drum slides upon a brass sleeve in bearings 1 cm. deep (to prevent "sidelash") and is held at any desired height by a spring clip. The sleeve ends in a friction plate which rests upon a metal disk driven by the clockwork. Sleeve and friction plate revolve about a steel shaft which passes through the heavy plate containing the clockwork, and is securely bolted to the bottom plate. The sleeve bears upon the steel shaft only by means of "bushings" at the end of the sleeve, thus securing a bearing without "sidelash" and with little friction. As the sleeve of the drum rests upon the friction plate by gravity alone it is easy to turn the drum by hand either forward or backward, even while the clockwork is in action. At the top of the sleeve is a screw ending in a point which, when the screw is down, bears upon the end of the steel shaft and lifts the sleeve, and with it the drum, until the sleeve no longer bears upon the friction plate. The drum may then be "spun" by hand about the steel shaft. The impulse given by the hand will cause the drum to revolve for about one minute. The speed during any one revolution is practically uniform.

The clockwork consists of a stout spring about 6 meters in length, driving a chain of gears. The speed is mainly determined by a fan slipped upon an extension of the last pinion shaft in the chain. Four fans of different sizes are provided.

The speed is regulated by a governor on the shaft that carries the fan. When the milled head shown to the right of the steel shaft in Fig. 138 is up, the gear on the extreme right no longer engages with the gear driven by the spring, but runs "idle," while the gear attached to the friction plate engages with the lower of the two gears shown at the left; the pinion of this lower left-hand gear engages with the spring gear. Fast speeds are then obtained.

When the milled head is down, as in Fig. 138, the gear attached to the friction plate falls below the left-hand gear, while the right-hand

[1] W. T. Porter: Introduction to Physiology, 1909, p. 79.

gear engages with the spring gear and through a pinion drives the friction-plate gear. Slow speeds are then obtained.

These operations are easily and rapidly performed, though, as in all mechanisms, an instant's pause is sometimes required to enable the gear teeth to engage. The clockwork should be in motion without the fan, when the adjustments are being made.

With both fast and slow gearing four fans of different areas may be used. They are slipped upon an extension of the last pinion shaft in the chain. Five slow and five fast speeds (exclusive of spinning) are thus obtained. An additional slow speed (50 cm. per hour) may be obtained with a very large fan.

Long Paper Kymograph.[1]—In Fig. 138 the kymograph is arranged for use with a sheet of smoked paper about 8 ft. long. A rigid bench of steel about 97 cm. long firmly supports two]-shaped frames in which two aluminum drums revolve on pointed adjustable bearings. The rear frame with its drum slides along the bench, and

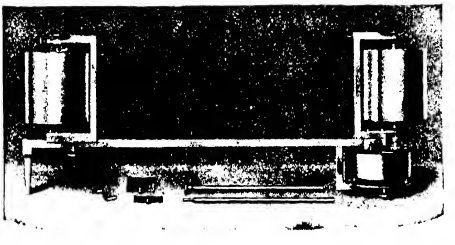

FIG. 138.—(Harvard) long paper kymograph. About one-twelfth the actual size.

may be fastened at any desired distance from the remaining or clockwork drum, so that paper from about 150 to 240 cm. in length may be stretched between the drums. The rear frame is provided with an adjusting screw with which the drum may be inclined until the strip of paper is stretched uniformly throughout its height. When the adjustment is complete the abscissæ drawn by a writing lever in successive revolutions will exactly coincide. The clockwork

[1] W. T. Porter, Science, 1906.

drum does not slide along the bench. Both drums may readily be removed from their bearings.

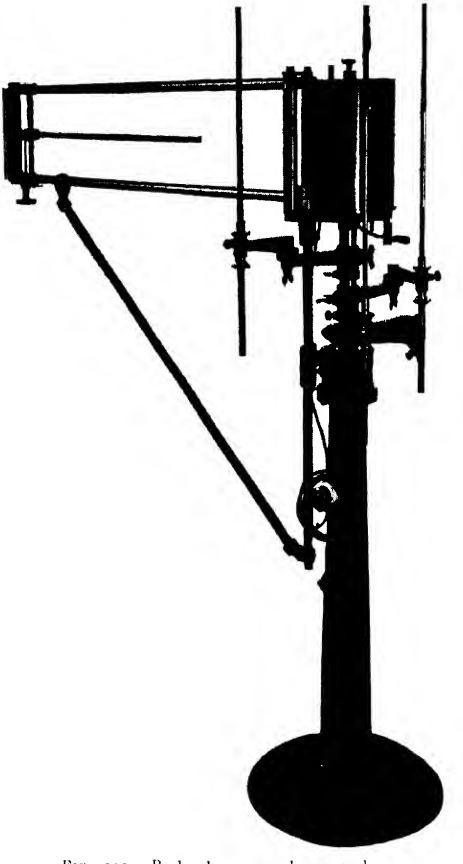

FIG. 139.—Becker long paper kymograph.

Beneath the clockwork drum is a circular plate of the exact size of that of the single drum kymograph. This plate rests on two feet and supports the anterior end of the steel bench (Fig. 138). Upon

the plate are three rounded pins. The clockwork drum is driven by a kymograph, the feet of which are hollowed to fit the three rounded pins just mentioned. When the kymograph is set upon these pins, it is at once "centered" and all side motion is prevented. The vertical steel drum rod and sleeve of the kymograph are replaced by a short rod with a sleeve ending in a collar, the top of which is flush with the upper plate of the kymograph. The collar is pierced with four holes. To connect the clockwork with the drum a coupling sleeve is let down from the shaft of the clockwork drum until four pins on the under surface of the coupler engage the corresponding holes in the kymograph rod.

The Becker kymograph shown in Fig. 139 is designed for research work but serves admirably for student demonstration. The motor which drives the kymograph is placed within the large drum, thus doing away with all troublesome belts and making it possible to mount the complete kymograph on a pedestal, the base of which is 20 inches in diameter. Four different speeds are available so that the full length (15 feet) paper makes one complete revolution in 2 3/4 hours, 1 1/2 hours, 1 hour and one in 15 minutes.

The kymograph may be raised or lowered through a distance of 18 inches. It is equipped with three adjustable rodholders and fine adjusting screw. Two of these holders move with the writing surface when the kymograph is raised or lowered. By means of an idle drum a flat writing surface is obtained. The rear drum is adjustable so that papers varying between eight and fifteen feet may be used. The paper is tightened by a rack and pinion.

Langenbeck's Forceps.—See Bulldog clamps.

Leg Bands.—Leg bands are especially servicible for numbering roosters, rabbits etc. They are usually made of aluminum or leather. The most commonly used types are shown in Fig. 140, and may be obtained from the Wahmann Mfg. Co. of Baltimore. They are supplied in the following types and sizes:

No. 1—Smith sealed leg bands; made in six sizes.

No. 2—Double clinch leg bands; made in six sizes for chickens and two sizes for pigeons.

No. 3—Leader adjustable leg bands; made in six sizes.

No. 4—Challenge bands; made in one size only.

No. 5—Open pigeon band; made in one size only.
No. 6—Colored leader bands made in one size only.
No. 7—Ilco celluloid bands.
No. 8—Ilco coil baby chick band.
No. 9—Moes celluloid bands.

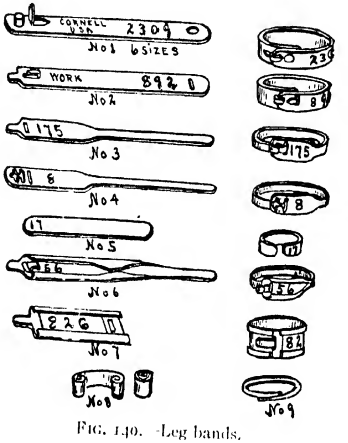

FIG. 140.—Leg bands.

Ligatures.—The ligatures should not be too thick and should be tied as tightly as possible. Linen thread or button-hole twist answers the purpose for vessels, but lacks sufficient strength unless a very coarse thread is employed. For this reason I find "dental floss" to be about the best cheap ligature, for vessel work, as it is both fine and of high tensile strength. Twine should be employed for trachea, bladder, etc.

Manometer.—The *Harvard* manometer (see Fig. 21 page 67) consists of a glass U-tube mounted upon a board to which is screwed a rod to be clamped in a stand. The one side of the U-tube is connected by a rubber junction to one limb of a glass T-tube. One of the other limbs of the T-tube is connected with a rubber pressure tubing long enough to reach to the carotid cannula. The remaining limb is connected with a rubber junction to a brass stopcock which in turn is connected by means of pressure tubing with a pressure

bottle containing 25 per cent. magnesium sulphate solution. The manometer is about one-half filled with mercury. The connecting tube and one side of the manometer are filled with magnesium sulphate solution by opening the brass stopcock. The end of the connecting tube is closed with a pinch-cock.

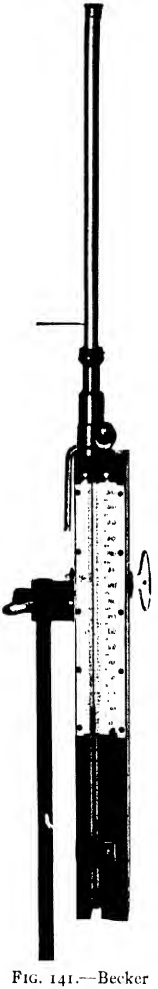

FIG. 141.—Becker manometer.

The other side of the U-tube bears a hard rubber float, for recording the excursions of the mercury. The float is hollowed to conform with the meniscus of the mercury and should fit the tube snugly but have sufficient play to avoid friction. The float bears a piece of aluminum wire about 20 cm. in length, well centered, which passes through the hard rubber cap at the end of the U-tube. To the upper end of this wire is sealed the writing style which is held against the drum by a guide consisting of a silk thread suspended from a rod and loaded with a small weight.

When measuring tracings it must be remembered that the real rise in blood-pressure is twice that which is recorded, since there are two sides to the U-tube and the needle only passes through a space that represents one-half of the difference of level between the mercury in the two sides.

The *Becker* manometer (see Fig. 141) is constructed of carefully selected calibrated, annealed glass tubing, which is mounted on a hard rubber block. The horse-hair and weight of the actual monometer are replaced by a slotted brass tube which serves as a guide for the writing needle, assuring continuous approximation of writing point to paper. The writing point is brought into contact with the paper by turning a screw which rotates the brass guide tube. The scale is not fixed, as in other types of manometer, but can be adjusted at "blood pressure zero" for

each experiment. The three-way cock serves to put into communication the anti-clot reservoir, the manometer and the blood vessel.

Moist Chamber. - The moist chamber (Fig. 142) is an ingenious device to prevent drying of the nerve and consists of a porcelain plate which bears near the margin a shallow groove. In this groove rests a glass cover which for the sake of clearness has been omitted from the figure. To the porcelain plate is screwed a rod, by which the plate may be supported on a stand. Within the glass cover are two

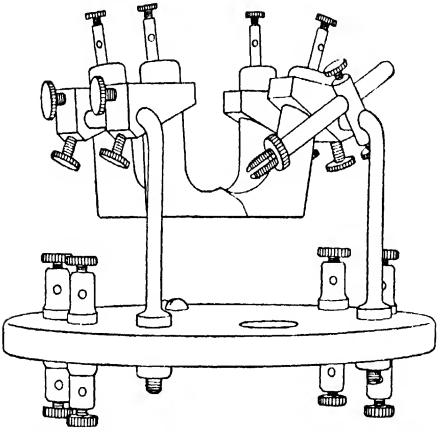

FIG. 142.—The moist chamber; about three-fifths the actual size. (From Porter's Introduction to Physiology.)

right-angle rods. One of the rods carries a small clamp, composed of a split screw on which moves the nut, by means of which the femur of the nerve muscle preparation may be firmly grasped. The holder for the split screw is arranged to permit of motion in all directions. Both right-angled rods carry unpolarizable electrodes. Each of these is borne by a hard-rubber holder. By turning the leg of the boot in the holder the foot may be brought as near the foot of the neighboring electrode as may be desired. These boots should be kept in normal salt solution. In use the hollow leg of the boot is half filled with saturated solution of zinc sulphate and placed in the

clip. To the hole in the zinc plate is attached a wire which connects with one of the four binding posts shown in Fig. 142. These four posts are in electrical connection with four posts beneath the porcelain plate. The electrodes are made of unglazed potter's clay and have the shape of a boot. The boot electrodes serve equally as well for leading off the nerves or muscles to the electro-meter and for stimulation. After use the boots should be emptied, rinsed with tap water, drained, placed in several hundred cubic centimeters of

FIG. 143.—Shows method of exposing saphenous and femoral veins: *a*, femoral vein; *b*, saphenous vein, showing its junction with the femoral; *c*, femoral artery; *d*, saphenous artery, showing its junction with the femoral.

normal saline solution until wanted again. *If the foot of the boot is kept saturated with normal saline solution, these electrodes will remain non-polarizable.*

The air within the moist chamber may be kept saturated with water vapor by applying moist filter, or blotting paper to the inner side of the glass globe.

Muscle Lever (Light).[1]—A stout yoke bears two set screws holding a steel axle upon which is mounted a light piece of tubing and

[1] W. T. Porter: First catalogue of Harvard physiological apparatus, September, 1901.

a metal pulley. One end of the tubing tapers slightly to receive the writing straw. The other projects behind the axle, and may be pressed upon by the accurately cut after-loading screw. The pulley is pierced with a hole for securing a fine wire by means of which a weight may be suspended from the pulley when it is desirable that the weight should be applied near the axis of rotation. The muscle may also be weighted by means of a scale pan suspended from the double hook to which the lower end of the muscle is attached. If the tendon of the muscle be fastened to the double hook by a fine wire, the free end of the wire may be carried to the insulated binding post provided for convenient electrical stimulation. The upper end of the muscle may be grasped in the flat-jawed clamp and thus connected electrically with the binding post upon it.

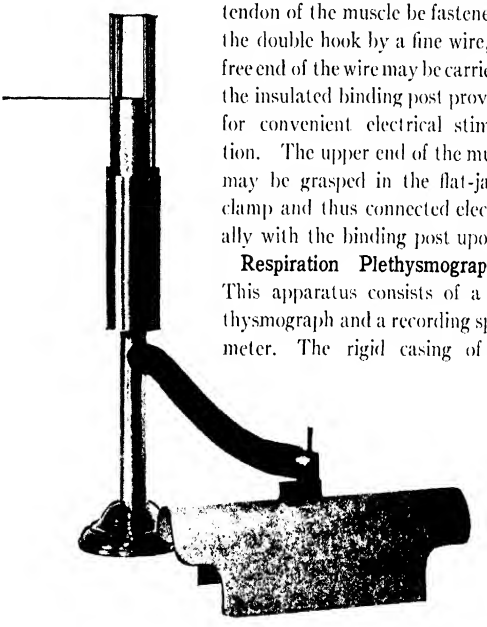

FIG. 144.— Becker respiration plethysmograph.

Respiration Plethysmograph. This apparatus consists of a plethysmograph and a recording spirometer. The rigid casing of the plethysmograph is semi-cylindrical in shape and is made of sheet steel. The free edges are flanged so that it may readily be fastened to the animal board. A thin-walled rubber bag is attached to the interior of the casing so that when the oncometer is placed in position it covers the entire animal except the head and neck. By means of

a wide, stiff-walled rubber tube the bag is connected with the spirometer. A heavy stand supports a cylinder which is filled with water before use. Within this is another cylinder which carries the writing point and which is in communication with the rubber bag of the plethysmograph. The vertical movement of this recording cylinder is insured by four guides. A positive pressure in the plethysmograph is obtained by injecting air through a special side tube. The apparatus is so calibrated that 10 c.c. of air move the cylinder 5 mm. in a vertical direction. (See Fig. 144.)

Saphenous Vein. *Method of Exposing.*—The femoral vessels may be felt pulsating just below Poupart's ligament on the outer edge of the stiff adductor longus muscle. The artery lies partly behind and external to the vein. The dissection is performed so as to expose about 1 in. of the saphenous vein at its junction with the femoral. (See Fig. 143.)

Signal Magnet (*Chronograph Harvard*).—This signal magnet consists of a small metal box (Fig. 145) open at the front and ends, enclosing a strong magnet, the armature of which is mounted upon

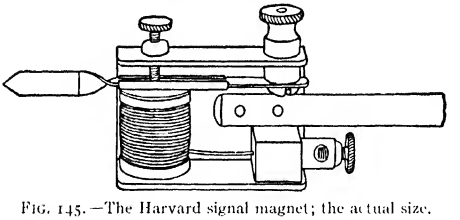

FIG. 145.—The Harvard signal magnet; the actual size.

a steel spring. An accurate fine adjustment screw regulates the excursion of the armature. One binding post is mounted upon the metal box, the other is insulated by a rubber block. This signal, in circuit with a vibrating tuning fork, will record 100 double vibrations per second. In the primary circuit of the inductorium it will record the make and break of the circuit without the after-vibration. The handle is long enough to bring the writing point directly above or below the writing point of the muscle lever clamped to the same iron stand.

"Lag" due to residual magnetism is lessened or prevented by parchment paper shellaced to the under surface of the spring over the core of the magnet. The paper should be renewed when necessary.

The signal magnet or chronograph is used in conjunction with a time marker (see Figs. 147 and 148) for making time tracings. The signal point records on the smoked drum the number of times per second a current through it is made and broken by the clock. A signal magnet is also employed in connection with drop markers etc. The writing point of the signal magnet is placed in under and exactly on a vertical line with the writing point of the manometer, heart lever or muscle lever as the case may be.

FIG. 146.—Becker signal magnet.

The signal magnet is also useful for marking the time of injections, stimulations, etc. The electro-magnet is connected with a battery, with the interception of a key, which is closed whenever a mark is to be made on the drum.

The *Becker* signal magnet is a distinct departure from the usual form of construction. The instrument consists of a brass tube $\frac{3}{8}''$ in diameter which contains a long electro-magnet. The writing lever is a celluloid tipped straw which slips into a slotted tube. The extent of the movement of the writing point can be regulated by adjusting the milled screw cap.

Simple Electric Key.– The simple electric key is used to make and break the electric current. It is especially useful in the study of the effect of stimulation on muscle and nerve muscle preparations. There are several forms on the market, but a small electric-light switch will answer for many purposes.

Tambour.—The Marey Tambour is a very simple but satisfactory instrument to be used in recording the intra-thoracic pressure curve,

or for any purpose for which a tambour of this type may be adapted. The moving parts are very light. The membrane may be made of "dental dam" or any similar rubber, not too tightly stretched.

Temperature of Animals.—This is practically always taken in the rectum. In order to secure uniform results the thermometer should always be inserted for the same distance, which may be accomplished by marking the stem.

Time Marker (*Becker*).—This time recorder was described by Professor C. C. Lieb in the Journal of Pharmacology and Experimental Therapeutics (vol. ix, 1916-17, 227). The second-hands of a watch are replaced by toothed geared wheels which activate a writing lever. Time may be recorded in minutes alone, in minutes and seconds, or in minutes and five-second periods.

The writing lever is made of straw; the writing point of celluloid. (See Fig. 147.)

The Harvard Electric Clock or time marker is used in conjunction with a signal magnet for making *time tracings*. The writing point of the signal magnet is placed in under and exactly on a vertical line with the writing point of the manometer, heart lever or muscle lever as the case may be. The clock at regular intervals makes and breaks the electric current thus causing the signal magnet to mark the time on the smoked chart.

Figure 148 shows the Harvard electric clock. "The pendulum bearing the armature is released by pressing down the bar at the right. With each swing the pendulum turns one of the several toothed wheels past an adjustable contact connected with the signal magnet at the physiologists' kymograph. This contact is made once in 1, 5, 15, 30, or 60 seconds, according to the toothed wheel used.

The five wheels are mounted side by side on a rod, the outer end of which is seen in Fig. 148. When the pendulum begins to "run down" the movable piece hung at the lower end pauses upon a plate borne by a spring between two magnet coils. Contact is thus made and the magnet now suddenly active pulls the armature, and with it the pendulum up to the full swing, thereby breaking the magnet circuit till the pendulum lags again. (See Fig. 148.)

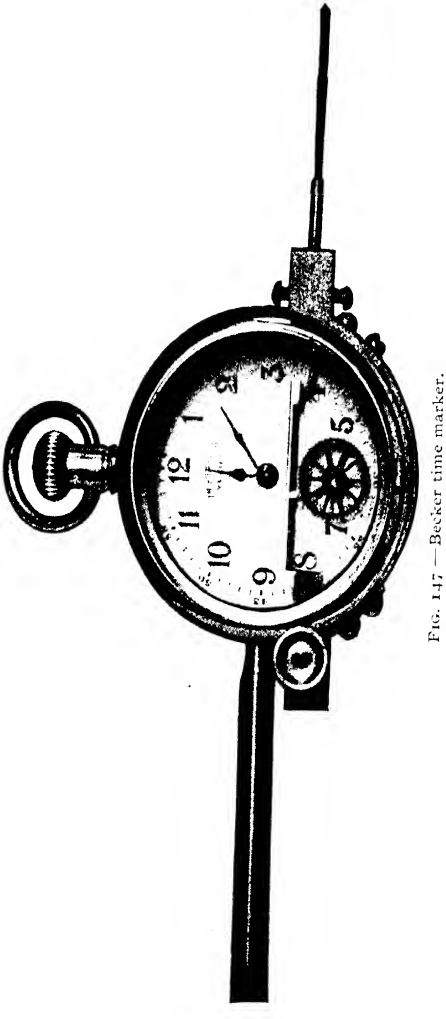

FIG. 147.—Becker time marker.

Tracheotomy.—Is the formation of an artificial opening into the trachea. Tracheotomy is necessary in all experiments on mammals

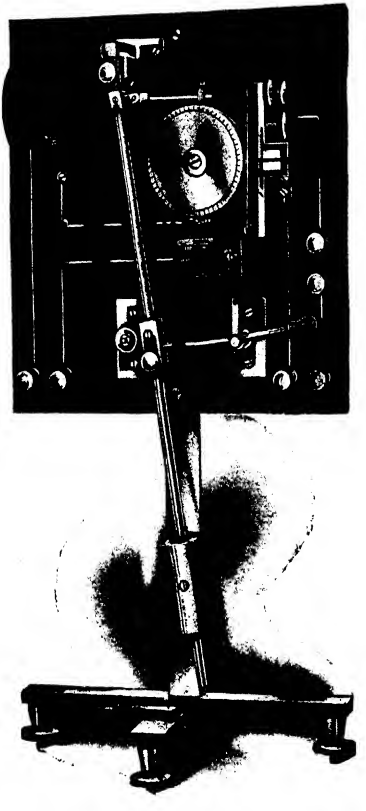

FIG. 148.—Harvard time marker (electric clock).

which require artificial respiration. The trachea is freed immediately below the thyroid cartilage and opened. A tracheal cannula (see Fig. 120) is then inserted and tied with a small stout twine.

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CHAPTER XX

SOLUTIONS

Crude Drugs.—Solutions of crude drugs for testing purposes should be made by percolating a small representative sample according to the official method for preparing the tincture or fluidextract of the drug under consideration.

Epinephrine, *Standard Solution* (1 *in* 10,000).

Standard epinephrine crystals	0.01 gm.
Dilute hydrochloric acid	1. drop
Normal saline solution	q.s. 100.00 c.c.

Ergot, *Standard fluidextract.* —See p. 219.

Extracts.—Powdered, solid or fluidextracts should be diluted with solvents of the same alcoholic strength as that used in the original extraction of the drug. This is necessary in many cases, to effect complete solution of the active constituents.

Locke-Ringer's Solution.

Sodium chloride reagent[1]	9.000 gm.
Potassium chloride reagent	0.420 gm.
Calcium chloride reagent	0.240 gm.
Magnesium chloride reagent	0.005 gm.
Dextrose reagent	0.500 gm.
Sodium bicarbonate reagent	0.500 gm.
Water, freshly distilled from a hard glass flask	q.s. 1000.00 c.c.

All the salts should not be added to the water at the same time as this results in a cloudy solution probably caused by the formation of calcium carbonate, but should be added and dissolved in the order named, in which case calcium carbonate dissolves in the sodium and potassium chloride solution resulting in a perfectly clear solution.

[1] Baker's "Analyzed" Sodium Chloride, *Special Crystals* seems to be the most satisfactory.

The solution must be made up fresh each day. The constituents (except the dextrose) may be made up in more concentrated stock solutions and diluted as needed.

Locke-blood Solution.—Add 5 to 10 per cent. of defibrinated whole blood to the above Locke's solution.

Normal Saline Solution.

For Frogs:

Sodium chloride	7.5 gm.
Water	q.s. 1000.0 c.c.

For Mammals:

Sodium chloride	9.5 gm.
Water	q.s. 1000.0 c.c.

Perfusion Solutions.

Aconite	0.1	per cent. in Ringer's Solution
Digitalis	1.0	per cent. in Ringer's Solution
Epinephrine	0.0001	per cent. in Ringer's Solution

Physiological Solution of Sodium Chloride.

Sodium chloride	8.5 gm.
Distilled water	q.s. 1000.0 c.c.

Pituitary, *Standard Solution.*—See directions given on page 220.

Ringer's Solution.

For Frogs:

Sodium chloride reagent	7.00 gm.
Potassium chloride reagent	0.30 gm.
Calcium chloride crystals	0.26 gm.
Water	q.s. 1000.00 c.c.

Ringer-Langendorff Solution.

NaCl	8.000
$CaCl_2$	0.100
KCl	0.075
$NaHCO_3$	0.100
Water	q.s. 1000.000

Solutions Used to Prevent Clotting.—In blood-pressure experiments the cannula and connecting tube should always be filled with one of the following solutions to prevent clotting.

1. Half saturated (25 per cent.) solution of magnesium sulphate.
2. One per cent. sodium citrate solution.

3. Half saturated (14 per cent.) sodium sulphate solution.

4. Half saturated (12 per cent.) sodium carbonate solution.

Care should be taken not to produce too great a preliminary pressure in the manometer, as ofttimes when opening the screw clamp on the connecting tube this high pressure forces some of the non-clotting solution into the heart. If this occurs prompt paralysis of the heart follows. The effects produced by small amounts entering the heart quickly pass off, larger quantities, however, stop the heart completely. When this occurs artificial respiration, normal salt solution injected into the vein, and cardiac massage, if started at once, will often resuscitate the animal.

In our experience we have found the magnesium sulphate solution to give the best results. The sodium sulphate and sodium citrate solutions are less dangerous but also less efficient. The sodium carbonate solution is about as efficient but is still more dangerous and is therefore not well adapted to this purpose.

Suprarenals, *Desiccated.*—See p. 68.

Tablets, powders, granulations, etc., should be finely powdered and triturated with a solution of the same alcoholic strength as specified by the U.S.P. or N.F. for extracting the drug for which the tablet is being assayed. For example, a tablet of digitalis should be triturated with a solution of the same alcoholic strength as the official menstruum for making Tincture Digitalis (Alcohol 4 parts, Water 1 part).

Tethelin.—Solution for intravenous injection in Blood Pressure test, see p. 132.

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CHAPTER XXI

RECORDS AND REPORTS

The activities of biologic control laboratories may be divided into two classes of work requiring two kinds of records and reports, *i.e.*, control assays and research or experimental problems.

Control assays include assays upon purchased items such as crude drugs, chemicals, etc., assays for controlling the various stages of manufacturing processes and the final assay and standardization of finished products.

Research or experimental problems include all experiments for the purpose of improving manufacturing processes, preventing deterioration, determination of physiologic actions of new synthetic compounds, etc.

The records of the results of research experiments carried out for the purpose of solving an experimental problem are usually much more voluminous than the records of results of routine control assays. This difference in volume and the fact that research records are seldom envolved in legal actions as is often the case with control assay results makes it advisable, in most laboratories, to adopt different methods for preserving the records of the two kinds of work.

Records and Reports of Routine Control Assays

The assayist must assume the responsibility for the identity, purity and strength of the ingredients before their use in the manufacture of the various products and also the responsibility that finished products are of standard strength and conform in every respect to label statements.

The records of control assays, therefore, must be kept in such shape as to be available at all times and unassailable as evidence in any litigation. For this reason, although loose leaf systems possess advantages in many modern business record systems, *they should*

not be used for recording routine control laboratory results. Each assayist should enter all such results, original calculations, etc., in bound record-books.

A loose leaf record is of little value as legal evidence as a leaf may be destroyed and replaced at any time. For the same reason the analyst should not make original notations, calculations, etc., on loose leaves and later enter them in a record-book. Such a procedure introduces the possibility of error in transcribing.

Each person entrusted with control assays should be provided with a **bound record-book.** The bindings of these books should be numbered consecutively for filing purposes and for added legal evidence as to the date of the entries as compared with the entries in other volumes of the same series.

For convenience the pages of the record books used for recording control biologic assays may be printed as shown in Fig. 149.

Each page may contain two headings, one at the top and the other in the middle of the page, in order to accommodate the results of two assays.

It will be noted that a heading of this type serves for practically all types of biologic assays. In the blank space in the first and third columns either Gm. or K. may be inserted depending upon whether the animal to be used is the frog, guinea-pig, rooster or dog.

In the case of frog assays for digitalis, strophanthus, squills, etc.; guinea pig assays for aconite, gelsemium and veratrum; cock's comb tests for ergot and cannabis assays the books should be used in the laboratories and *all entries made directly into the book.* As each animal is weighed the weight should be recorded in the book and not upon a piece of paper to be copied later. In other words the book should contain the **original** records of all control assays.

Directly beneath the assay results all calculations should be made shewing the strength of the product as compared with the standard. The person who conducted the assay should *sign and date the record.*

ALL ENTRIES SHOULD BE MADE IN INK

In the case of assays of pituitary extract, epinephrine, etc., where the results are recorded in the form of kymographic tracings, the tracings should be consecutively numbered, dated and inscribed with

NUMBER .....
TEST .....
PREPARATION .....

Dose per	Dilution	Dose of dil. per	Description or No. of animal	Weight	Actual dose of dil.	Time	Date	Results

FIG. 149.—Sample page from control assay "Record-book."

the name and laboratory number of the preparation *before the chart is "fixed."* They may then be filed numerically.

Each assay of this type should be entered in the record-book. After recording the name of the product, laboratory number and the nature of the test employed, an entry similar to the following should be made:

PHYSIOLOGICAL LABORATORY

No.....

.....192

Drug.....No.....Received.....

Test employed.....Standard.....

....

....

....

Amount used to produce desired effect.....

Per cent of activity as compared to standard.....

NOTES:

Signed.....

FIG. 150.—Sample page from laboratory report book.

"The above product was tested according to the U.S.P. *X* method. The results of a series of ten doses administered to the isolated uterus of a virgin guinea-pig show that the above preparation possesses an activity corresponding to 120 per cent. of the U.S.P. standard. Standard used—U. S. Bureau of Chemistry "Standard Pituitary Powder" No. 162. See kymographic tracing No..... Tracing record-book No. 210 page 146.

The assayist should enter each sample in the record-book *in the order received* regardless of laboratory or control numbers.

In large laboratories each assayist should be supplied with a "Report-Book" for transmitting assay results to the laboratory Director. A sample page from such a laboratory report book is shown in Fig. 150.

Experience has shown that books containing white printed pages for twenty five original reports and twenty five yellow or pink printed pages for carbon copies are the most convenient. The white pages

LABORATORY INSTRUCTIONS NO. 1356

BIOLOGIC ASSAY NO. 102

LAB. NO. 106, 757

PREPARATION Tr. Cannabis.

The above preparation when biologically assayed according to the U.S.P. method shows an activity of 66% of standard. Concentrate in vacuum to 50% of the present volume and resample.

John Brown DIRECTOR.

DATE Feb. 21, 1928.

FIG. 151.—Sample laboratory instruction sheet.

should be perforated so they may be easily detached. When an assay is completed and the records entered in the record book the assayist should record the required information in the laboratory report book and forward the original to the laboratory Director.

The Director with his knowledge of the "Interpretation of Biologic Assays" (see Chapter XV) issues "Laboratory Instructions" based upon this report to the manufacturing departments for the dilution, concentration or release of the product in question.

BIOLOGIC ASSAY NO.	LAB. MFG. OR FIN. NO.	PREPARATION	REC'D FROM	TEST	ASSAY RESULTS	INSTRUCTIONS
101	106,743	TINCTURE DIGITALIS	Fluid Dep't	U.S.P.	M.S.D. = 0.004 Ouabain at 0.000,0005	150% Dilute 25% and Resample
102	106,757	TINCTURE CANNABIS	Fluid Dep't	U.S.P.	0.45 cc per K. = Ataxia	66% Concentrate and resample
103	106,762	TINCTURE SQUILLS	Fluid Dep't	U.S.P.	M.S.D. = 0.004 Ouabain 0.000,0005	100% O.K.

FIG. 152.—Sample page from Laboratory Director's "Key Book."

A typical "Laboratory Instruction" sheet as issued by the laboratory Director is shown in Fig. 151.

Both the laboratory report and the Directors instructions to the manufacturing departments are filed numerically according to the biologic assay number.

The laboratory Director's system of records should include first a "Key Book" for consecutively numbering and entering each sample received for assay. The author uses for this purpose a book in which each page is divided into seven vertical and 15 horizontal columns. The horizontal columns are about one inch wide and consecutively numbered. The vertical columns are "headed" and entries made as shown in Fig. 152.

After entering each preparation received in the above book, which lists the samples consecutively according to biologic assay numbers, they are card indexed according to laboratory number and the name of the preparation. This method provides a ready means for future reference.

The key book contains only the summary of results and instructions. The complete instructions and the laboratory report upon which the instructions were based are always available from their respective files. The assay results showing the effect of each individual injection, etc., is always available in the laboratory record-books.

The complete laboratory record of a product assayed may, therefore, be traced at any time from either the biologic assay number, manufacturing number or finishing number.

RECORDS OF RESEARCH OR EXPERIMENTAL PROBLEMS

It is just as important to keep accurate carefully filed records of seemingly unimportant experiments as of the major activities of the laboratory. It frequently happens that the supposedly unimportant experiments oft times suddenly becomes of major importance. In such cases if the original results are not carefully recorded and preserved it becomes necessary to repeat the experiments in order to obtain accurate data. The results of **every experiment that is worth performing are of sufficient value to record and rpeserve.** Very often the best research worker is the poorest systematizer

as far as records are concerned. It is not sufficient to simply keep accurate records of all experimental results in note books or miscellaneous files. These records should be filed and indexed in a manner in which they will be available for future reference.

The larger the laboratory organization the greater is the need for a definite record system, and the necessity for all workers keeping records in the same manner. If each worker in a large laboratory keeps records according to his own individual ideas it often happens in the case of death or change of position that the results are entirely lost or it is impossible for others to locate or interpret them. Such conditions necessitate the repetition of experiments with the incidental additional expense and loss of time. Likewise with the individual method of keeping records it ofttimes occurs that work is unknowingly duplicated by workers in different laboratories of the same institution.

The records of the laboratory should be so systematized that with little labor a research worker may obtain the *complete record and summary of results* of all previous experiments bearing upon the problem he has to solve.

The following system will be found adequate for handling the records of from one to any number of research workers or laboratories.

Depending upon the volume of work a single correspondence size drawer, a four drawer cabinet or a series of such cabinets should be kept in the Laboratory Director's office and designated as the *Experimental Problem File.* Each investigation, whether it consists of a single test or an extensive series of tests, should be given an *experimental problem number.* These numbers should be given consecutively in the order in which work upon each problem is started. If the maximum value is to be derived from research work the director should inforce the rule that *work of an experimental nature must not be undertaken until the problem has been assigned a number.*

If the problem originates in the directors mind the number should be assigned and the experiment authorized before the work is given to the research worker. If on the other hand the problem originates with the worker he should outline the experiment and then obtain

the experimental problem number, additional instructions, authorization, etc., from the director before beginning the work.

As soon as the number is assigned to a problem a folder is started for filing the results. The folders are "headed" with the "Experimental Problem Number," the name of the substance under test and if possible the motive of the test.

Suppose, for example, the first problem is to determine the method of excretion of Hexylresorcinol in rabbits, cats and dogs. In this case the folder would be "headed" as follows:

NO. 1 Hexylresorcinol
Excretion in rabbits, cats, dogs.

A brief outline of the experiment which should include any special instructions should then be prepared in duplicate, one copy of which is kept by the worker to whom the problem is assigned and the other is placed in the folder which is then filed numerically.

Each experimental problem is indexed numerically and according to the name of the substances under test.

In the case of the above example one index card should read:

No. 1 Hexylresorcinol

To determine the method of excretion in rabbits, cats and dogs.

Assigned to W. J. Black.

The other index card should read:

Hexylresorcinol Exp. Prob. No. 1

To determine the method of excretion in rabbits, cats and dogs.

Assigned to W. J. Black.

In large laboratories the Director may require an additional index card to be placed in the calendar file for "follow-up" purposes.

Each research worker may then keep his records according to his individual fancy but *duplicate outlines of all experiments and experimental results must be forwarded for filing in the "Experimental Problem File."* If the results of any experiment become too voluminous for a folder they are removed and placed in a transfer box which is numbered and labeled in the same manner as the folder.

Whether the system is used by a single worker for filing the results of his personal experimental work or is applied by a laboratory director for filing the records of a series of research laboratories, *a "Summary of Results" should be prepared immediately upon completion of each experimental problem.* In other words the first sheet in each folder should contain the outline or object of the experiment and the last sheet should contain the "summary of results." Such a summary can usually be prepared with little effort while the details of the experiment are fresh in the mind of the experimentor whereas in many cases, in the absence of such a "summary," it may be necessary for another worker to study the mass of results for days or weeks in order to properly interpret them and prepare a "summary."

The preparation of such a summary usually does not involve additional labor as in most cases the executives of the institution of which the laboratory is a part are not interested in all of the intricate details of the various experiments and their results. They are interested in the summary of results or in other words the conclusions to be derived from the experiments.

The summary of results should, therefore, be prepared in duplicate *immediately upon completion of a problem.*

The original is used to "close" the experimental problem folder while the copy serves as a basis for the "conclusions" in the directors official report.

The report on the problem given above should read somewhat as shown in Fig. 153.

It will be noted that with an index of all the substances studied and each experimental problem folder containing a "Summary of Results," it is possible for any member of the research staff to

obtain a summary of all previous experiments which have been carried out upon the substance in which he may be interested.

EXPERIMENTAL PROBLEM NO. 1

HEXYLRESORCINOL

To determine the method of excretion in rabbits, cats and dogs..

Groups of twenty-four rabbits, twenty-four cats and twenty-four dogs were each divided into two lots of twelve animals each.

The Hexylresorcinol was administered to one lot of each group by stomach tube and to the other lot by intravenous injection. A study of the various methods of excretion showed that most of the substance administered was in all cases excreted by the urine. Some of the substance was also shown to be present in the bile.

"Conclusions"

Hexylresorcinol is excreted principally by the urine.

March 2nd, 1928. John Brown
DIRECTOR.

FIG. 153.—Sample report on special or experimental work.

Such a system, therefore, will fill the requirements of any size laboratory for a means of obtaining information in detail or in the

form of a summary of all previous work and thus eliminate duplication of efforts and avoid the loss or the possible inability to interpret records of former employees.

Both of the systems described above may of course be elaborated to meet special requirements but in the interest of economy and exactness every routine or research laboratory should have systems at least as comprehensive as the ones outlined.

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INDEX

A

G

H

M

N

O

S

T

U

V